

Karl Georg Wingstrand

The Structure and Development of the Avian Pituitary

C W K GLEERUP / LUND

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The Structure and Development of the Avian Pituitary

From a Comparative and Functional Viewpoint

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Abbreviations

d = days, used to denote the age of chick embryos.

CR = crown-rump length, used to denote the size of embryos.

TL = total length, used to denote the size of embryos.

R = Romeis (1948), referred to for histological methods.

Introduction

During my anatomical studies on the pituitary which started in 1943 I have collected a fairly extensive material of section series and other anatomical preparations. The results obtained from reptilian material will be published later. The observations made on avian material will be presented separately here.

Originally, I did not intend to write a monograph on the bird pituitary, but devoted my interest to some special problems. First I investigated the histology of the glandular cells of the pars distalis in order to get a reliable basis for experimental research. I admit that I was not very successful with this part of the work, because of technical difficulties offered by the material. Furthermore, the complicated conditions which I observed in some reptiles which were easier to interpret made me doubt my own results. Later the embryological investigations and the Bodian technique in combination offered new possibilities for an objective interpretation of the glandular cells, and I believe some observations will carry the state of knowledge one step further.

The next problem which aroused my interest was the pituitary-thyroid relationship in the embryos, and the examination of three different species proved valuable for a more reliable conclusion. This was especially the case when the Bodian technique was applied for demonstration of the granules in the pars distalis of embryos. I then also devoted some time to the question whether the histological bipartition of the pars distalis had its origin in some bipartition of the hypophyseal rudiment in embryos, as proposed by Rahn (1938), but contradicted by other authors, and to questions of homology with other vertebrates.

Later I realized the important relation between the system of portal vessels in the pituitary and the function of the gland, and this work became still more stimulating after I had seen Harris' and Green's excellent papers on these problems. This part of the investigation required a detailed examination of the neurohypophysis and the vascular system and I soon realized that these structures had to be followed from their origin in embryos if their interrelationship should be understood.

When this last part of the work had been finished and some complementary investigations on the innervation of the pituitary had been made I looked over my collections of slides and found that only little had to be added if the work should be made a monograph on the structure of the avian pituitary. Though some of the chapters thus added do not contain any fundamentally new view-points, my

material has allowed me to give the previous statements a more general validity within the avian group. In other cases some results gained from animals of other classes have been applied to birds for the first time. I then thought that a monograph was the best way for summarizing all the results. Thereby all the observations are put in relation to each other and can be discussed without unnecessary repetition of facts.

The great homogeneity within the avian class as regards anatomical features of the pituitary does not justify a separate description of the pituitary in each species. I have therefore in separate chapters described the appearance of each anatomical division of the gland, and then I have usually chosen to describe the type of the organ present in the majority of birds. If essential deviations from this general type have been observed this has always been stated.

In my descriptions I have laid special stress on the structures which may be important either for the comparative morphological interpretation of the gland and its surroundings or for the understanding of the function. I admit that the function of the gland can only be understood completely from physiological experiments. Yet I have sometimes included some functional aspects in the discussions. This usually means that I point out the possibilities which must be considered when the function is to be interpreted, and only rarely can these possibilities be reduced to one. The definite choice between the possibilities will be the task of the experimental physiologist.

The literature concerning the pituitary gland in general is enormous and I admit that I have not seen all of it. In this paper I have preferably confined myself to the literature on birds, and I have mainly considered more modern works. The numerous papers on the development and structure of the pituitary written in the 19th century have usually only historical interest. These earlier works will be found in the literature lists published by Tilney (1912), Stendell (1914) and Bruni (1914).

Much confusion and many unnecessary discussions have been caused by the failure of some investigators to distinguish homologies from analogies, and it is true that this is difficult in practise. In my discussions on homologies I have therefore made considerable efforts to avoid criteria which are intimately connected with the function of the gland. The concept homology is used in a strictly morphological sense to denote morphologically comparable structures in different animals, i. e. *to denote corresponding units in their common structural pattern*. The early embryonic development of the structures is regarded as the most decisive criterium in all cases, since it is highly independent of the functional specializations in the adult. The position of a structural part and the relations to other structures in the adult are regarded as a second-hand criterium. Histological and functional features are regarded useless for discussions of homologies between far-related species, since extensive variations in these respects may be found in closely related species of some groups. Once homologies have been established, the next step is a comparison of the homologous parts from a functional and histological point of view. It must be emphasized once more, however, that such comparisons must be clearly distinguished from homologization.

Acknowledgments

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I am afraid no member of the staff of the institute has escaped being attacked with questions concerning my work. In fact, sometimes I have felt afraid that I have troubled my colleagues too much with my problems, but their readiness to criticize my ideas and to hear my lamentations on unsuccessful experiments has been phantastic. As I clearly realize how many mistakes I escaped in this way and how inspiring these discussions have been *to me* I keep all my colleagues in grateful memory, though it is impossible to mention all of them here. Of scientists outside the Institute I want to mention particularly Dr Helge Volsøe, Copenhagen, with whom I have spent many evenings discussing anatomical problems, and who also supplied me with valuable material. I have also had the luck to discuss the physiological problems involved in this investigation with Dr Dora Jacobsohn, Lund, Dr G. W. Harris, Cambridge, and Dr N. Å. Hillarp, Lund, and this was especially valuable since they are all interested in the same problems as I am.

The members of the Lund University Chile Expedition, professor Hans Brattström and Dr Erik Dahl, have contributed to the present work by collecting material of South American birds. In fact, 18 of the species examined were secured and preserved by the expedition and many other specimens are left for further research. The value of this material is increased by the excellent state of preservation of the specimens and by the interesting systematic position of the species. I thank the members of the expedition for their time-consuming work with the material, and I also want to thank Dr Dahl particularly for his readiness to collect and preserve pituitaries of Swedish birds for me.

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CHAPTER I

Material and Methods

Material of Adult Birds

As the present work has a monographic character it must of course be based on a material really representative of the class Aves. I maintain that this condition is satisfied as regards the gross anatomy and most histological aspects of the pituitary. Table 1 (p. 18), which shows the material of adult birds, contains 69 species belonging to 18 orders of birds from the Struthioniformes to the Passeriformes (system according to Berlioz 1950). In fact, only 9 orders of birds are not represented, and considering the great homogeneity in the gross structure of the avian pituitary I do not think we can expect any important deviations within the non-investigated groups. In fact, I have also sectioned some embryos of *Fulica atra* L. (Ralliformes) which show that the pituitary in this species is developed in the same way as in other birds, though, unfortunately, I have no adult material of this order.

I must admit, however, that the material is fairly heterogeneous in some respects. Most specimens had to be collected in the field, partly by myself and partly by other zoologists. This made it difficult to treat all specimens in a homogeneous way, but in fact I have seen little variation in the material which could be attributed to these circumstances, apart from some specimens which I knew were less perfectly treated. Further it was impossible to use all specific methods for every bird species. Injection of the vascular system, for instance, could not or only rarely be made if the bird was shot, and different fixation and staining methods require many specimens, which were often not obtainable. I have, however, tried to complete my material in such a way that, for every structure of importance, I could examine carefully at least one representative of each order of birds. Thus I have tried to get, for each order: 1. one series of celloidin or paraffin sections with the gland in situ, which shows the true shape of the organs, the surrounding structures and the vascular system. 2. At least one series of thin paraffin sections stained in azan, which allows a study of most histological details. As can be seen in table 1 this ideal was arrived at for most of the investigated orders.

On the other hand, silver impregnation for demonstration of nerve fibres and argyrophilic granulations in the glandular cells (according to Bodian) was not

applied for representatives of every order, for I realized the value of this method fairly late. So many birds were, however, examined by this method that I think the material will suffice for general conclusions (see table 1). The investigation of the hypothalamic nerve fibres had to be confined to a few species, however.

Other specific methods also proved valuable, but the results mainly confirm the statements made from the routine azan preparations. If they have been tried only for a few species of which I had abundant material this cannot weaken the conclusions in any essential degree. The Gömöry technique for demonstrating reticular fibres, for instance, was applied only in 6 cases (5 species). The reticular membranes proved to be arranged in the same way as in mammals, and I did not consider it necessary to repeat the technique for more birds, the more so as the membranes can be observed also in azan preparations.

The possibility of individual variations within the same species is a source of error which I have had to consider particularly, because most species are represented in my collection by one or a few specimens only. However, it must be admitted that for a general review of the structure of the pituitary of birds it is better to base the descriptions on 180 birds belonging to 69 species than to deal with only a few species present in many specimens. On the other hand, the material of some species is large enough to eliminate also individual variation and, moreover, as several representatives of each order were usually examined, this will reduce the risk of mistakes concerning the taxonomical value of the observations.

The annual and diurnal variations as well as sex and age differences have been very little elucidated in this work. The material of each species is too small for conclusions concerning these problems. When labelling the preparations I have, however, noted also the sex, the age (as far as it could be stated), the date, and often also the hour. Differences caused by such cyclic and sex variations are, however, visible mainly in the glandular cells of the pars distalis and the complications caused have been considered when this part of the gland was examined.

Fixation, Sectioning, Staining

I can not enter into a detailed description of all fixation and staining methods here but refer to the manuals, preferably to Romeis (1948), abbreviated "R". Some technical methods will be described and commented on later in the text, and I here confine the survey to the routine methods used more generally.

Preparation. The specimens were preserved fresh, within two hours after death but usually within a few minutes. Some preparations left for a longer time after death were used for studies of gross anatomy only. Eyes, lower jaw, beak and the dorsal parts of skull and brain were removed and the specimen was put directly into the fixative. Of large bird species I usually preserved only the sphenoid region including the sphenoid bone, the pituitary, and the adjacent

parts of the brain. If a detailed study of the pars distalis was intended, I dissected out the gland before fixation, and this dissection took place in the following way (cf. also Putzig, 1935):

The preparation begins with removal of skin, lower jaw, eyes and upper beak. Then the rostrum sphenoidale is cut off so the pneumatic sinuses of the sphenoid become visible. In most birds the sella turcica and the tubes representing the carotic channels can then be readily observed in the sinuses. In a further step the muscles and bones are removed so that only the thin osseous lamella which forms the internal wall of the sphenoid and directly covers the brain and the pituitary is left. This preparation is made with a tweezer and strong scissors. The following manipulation comprises the removal of the thin inner wall of bone and is the critical point of the preparation. The pituitary itself is easily laid bare, but the bone tubes and lammellae at the exit of nerves and around the pituitary stalk are difficult to detach, because they are intimately connected with the dura. The connective tissue membranes must therefore continuously be cut free from the pituitary itself with a pair of pointed scissors. In this way it is usually possible to dissect out the pituitary with the stem, the eminentia, the pars tuberalis and the adjacent parts of the brain intact. Small dislocations of the different parts are unavoidable if the specimen is fresh. In many cases I preserved the gland in situ and dissected it out after the specimen had been transferred to alcohol. Then of course the different parts remain in a more natural position if the manipulations are made carefully.

Fixation. Most material was fixed in Bouin's mixture (R § 305) because the preparations can be left in this solution for a fairly long time, and this is valuable for field work. In some cases the specimens were treated with alcohol and embedded within a day or two after fixation, but also preparations which were left for a year or two in the Bouin solution proved useful for most purposes. The stainability had, in such cases, decreased a little and silver impregnation of nerve fibres was more difficult, but the changes in the cell structures were usually not considerable.

Some routine material was also preserved in Susa (R § 344), Zenker (R § 336), Helly (R § 337) or in 10 % formalin. The Susa preparations often gave a better differentiation of the basophils of the anterior pituitary than those preserved in Bouin and Helly, but the staining of the acidophils seemed to be suppressed and the differences were not very great. Formalin was used only in a few cases for specimens which were impregnated according to Bodian, but fixation of details is bad and the impregnation is not much better than after Bouin fixation.

Decalcification and trimming. Small specimens left in Bouin for some months were decalcified by the picric and acetic acids. If large specimens were to be cut in situ I used nitric acid (conc. acid : water = 6 : 94) after hardening in 96 % alcohol (R § 1613). The blocks were trimmed so that the even surfaces formed right angles, and the sections were cut perpendicularly to some surfaces. This makes the graphic reconstructions easier and more reliable.

Embedding and cutting. For detailed histological examination the dissected pituitaries were embedded in paraffin and in most cases I cut a continuous series of sections through the organs. The thickness of the sections varied between 2 and 12 μ , but usually did not exceed 6 μ . The thinnest sections, 2—3 μ , rarely permitted a continuous series, and the thickest (8—12 μ) were cut when the specimen contained hard bone or should be used for nerve impregnation. Also the entire

head of small birds could be successfully cut in paraffin. As a rule, however, I avoided paraffin sections of glands in situ, for the distortions will then often be great and many structures are destroyed by the unequal shrinkage of bone and soft tissue.

For examination of surroundings and vessels, also in ink-injected specimens, I used the celloidin method, which causes less shrinkage and makes the cutting of decalcified bone easier (R § 450). Large preparations were often left for months in the celloidin-alcohol-ether solutions. The thickness of the sections was 40 or 60 μ , for injected specimens up to 200 μ . It is often necessary to use so thick sections for else the number of slides will be too great, and the staining method described below demonstrates clearly also delicate structures in these thick sections.

Most specimens were cut sagittally, but I also checked the results in transversal and horizontal sections. As most structures of interest are unpaired and median the sagittal series give the clearest pictures and reconstructions can be avoided.

Mounting of the celloidin sections. It is very difficult and time-consuming to stain celloidin sections as thick as those prepared (40—200 μ) one by one, and they are almost impossible to mount on slides in the common way. I therefore worked out the following method for fixing them to the slide:

- 1) Place sections in order on the slide, tight together and wettened with 70 % alcohol.
- 2) Pour off excessive alcohol and press sections firmly to the slide with a double filter paper. If they are not dry repeat the procedure with a second paper.
- 3) 4 % celloidin solution (in ether-alcohol 1:1) is pencilled on the interspaces between the sections and on their margins, and also on the under side and on the edges of the slide.
- 4) Place the slide in 70 % alcohol to coagulate the celloidin. The sections are now included in a continuous sheath of celloidin which surrounds the slide.
- 5) If, during the following treatment, the plate with the sections should loosen from the slide the sections are never disordered but stick together firmly. It is then very easy to put back the celloidin plate with the sections on the slide, dry with filter paper and repeat the treatment with celloidin. Staining see next page.

Staining paraffin sections. The routine staining method was azan (R § 1489). Often a few slides of the series were impregnated with silver according to Bodian (R § 1816) for nerve fibres and argyrophilic granules of glandular cells. Some series, especially of geese, pigeons and ducks, were entirely impregnated according to Bodian or Palmgren (1948) for a complete study of the innervation. In table 1 I have indicated how many specimens were examined by the Bodian method.

Often the silver impregnation of nerve fibres according to Bodian was followed directly by azan staining. This procedure made it possible to produce simultaneously both nerve fibres, connective tissue and glia elements in the neurohypophysis. The best result of this combined staining was obtained after fixation in the alcohol-formalin-acetic acid mixture recommended by Bodian (80 % alcohol, 90 cc + 40 % formalin, 5 cc + glacial acetic acid, 5 cc). The staining in azo-carmin had to be prolonged for about 2—3 hours after this fixation, and the differentiation in aniline-alcohol had to be short. Also the time of treatment in the aniline blue — orange G — mixture had to be short, usually about 15—30 minutes.

A number of other silver impregnation methods for nerve fibres were tried a few times, but did not prove superior to the Bodian method. They are referred to in chapters X and XI. The impregnation of reticular fibres according to Gömöry (R § 1533) proved valuable and was used on slides from six series. A great many other staining methods were tried on single slides, but did not prove superior to the azan method or were valuable only for special structures. The most important were: Krezazan of Romeis (1948, § 2198) and acid fuchsin-aniline blue of Martins (1933) for pituitary glandular cells, phosphotungstic acid haematoxylin of Mallory for nervous tissue (R § 1874), Chromic haematoxylin-phloxin of Gomori for posterior lobe colloid (Lillie, 1948, p. 96—97), and for studies of gross anatomy also haematoxylin of Ehrlich with acid fuchsin or eosin as counterstains.

Staining celloidin sections. The staining of the thick celloidin sections caused me trouble at first, but the phosphotungstic acid haematoxylin of Mallory proved to be an ideal stain.

After washing in 70 % alcohol (a few drops of aniline oil added!) till the picric acid is completely removed the slides with celloidin sections are run through 30 % alcohol to water. Then staining takes place in the following way: 1) Phosphotungstic acid haematoxylin of Mallory (R § 685) 1—3 hours. 2) Wash in distilled water, several changes, till the celloidin is decolorized, usually about 1—2 hours. 3) Running tap water 5 minutes. 4) Dry with filter paper and run through two changes of 96 % alcohol, 5—10 minutes each. 5) Flatten and dry the sections with filter paper again, transfer to toluene, 100 cc + phenol, 10—20 g. Leave sections in this solution till they are transparent. 6) 2—3 changes of pure toluene. 7) Flatten sections once more with a filter paper, dip the slide in toluene and mount in balsam.

The result is a little variable depending on different fixation, decalcification etc., but it gives invariably a very clear, differentiated staining and the presence of the celloidin does not disturb the process. The nuclei, blood corpuscles, and some nerve bundles become blue, most nerve fibres stain in violet tones and connective tissue, bone and muscles stain in different shades of red. The result is of course not identical with that after the original schedule of Mallory, but I have found no stain which, under these conditions, gives an equally good result. The blood corpuscles and the connective tissue are so distinctly stained and the sections so transparent that this method allows a detailed study of small vessels also without injection of ink or starch.

Vascular injections. The injection of the vascular system of the head was made under profound ether anaesthesia either from the point where the carotis emerges from the neck musculature just behind the mandible or from its origin in the brachiocephalic trunk. In the latter case the pectoral muscles were loosened from the carina and drawn aside and the sternum was removed. The ink injections were performed with a syringe holding 5—10 cc. Undiluted india ink of common trade marks was used, and the injections were continued until abundant ink appeared in the jugular veins which were opened. The carotis of the other side was ligated during the injection.

The injection of starch-cinnabar solution for demonstration of the arterial system was made in the same way, but towards the end of the injection the pressure was increased considerably in order to fill also the smallest arteries. Of course no injection mass appeared in the veins in this case. If the injection mass is prepared

carefully in the following way the size of the starch grains permits them to pass into the small arteries, but not to pass the capillary nets (from Löwegren 1939, slightly altered):

I. Equal parts (by weight) of vermilion (mercuric sulphide), sifted through tight gauze, 5 % formalin, and glycerol are mixed in a mortar after the vermilion powder has been wettened with a little 96 % alcohol. This stock solution may be stored.

II. Immediately before the injection mix 4 parts (by weight) of rice starch, sifted through tight gauze, 5 parts of 5 % formalin, and one part of the emulsion prepared under point I. The mixture is filtered through the gauze, one or two times.

It is recommended to inject some 4 % formalin before the injection of the starch-cinnobar. As soon as injection is stopped, tie firmly a strong ligature around the neck of the bird, in order to prevent the fluid from returning. Preserve in formalin, 10 %. The specimen may be used for dissection, or embedded in celloidin and sectioned, or may finally be cleared up in benzyl benzoate after decalcification and dehydration, and examined in toto.

Material of Embryos

The investigation of the embryology of the pituitary was confined to three species of birds, viz. *Gallus gallus*, *Larus ridibundus* and *Riparia riparia*, which represent three different orders. As the study of the histogenesis of both neuro- and adeno-hypophysis required some specific methods, the technical work would have been too extensive if more birds should have been included. The embryos used are listed in table 2 (p. 21). I have, however, a number of embryos of other species in my collection to which reference will be made in the text, and in addition, I have looked through parts of the collections of the Tornblad Institute, Lund.¹ In neither of these other species did the material allow a study of the morpho- and histogenesis simultaneously, but on the other hand, I found no considerable deviations from the conditions in the three species described in this paper.

As a measure of the developmental stage I have used the "Crown-rump length" (CR) for small embryos and the total length (TL), that is the length from the point of the bill to the posterior end of the rump, when the embryo is stretched out, for older ones. I admit that these measurements are not quite safe for determining the stage of development, but they may suffice at least for an approximate classification of the embryos of the same species. For *Gallus* I used the time of incubation. The temperature of the incubator was about 39° C.

Most embryos were preserved in Bouin's mixture (R § 305) and a smaller number in other fixatives, e. g. Susa (R § 334), Zenker (R § 336) and Helly (R § 337). The small embryos were preserved in toto, the old ones were decapitated and the lower jaw and the eyes removed. In nearly full-term embryos the skull was opened dorsally.

For staining the sections of embryos I mainly used the same methods as for the adult specimens. The routine method was also here the azan-staining (R § 1489),

¹ I take the opportunity, here, to thank professor G. Glimstedt, the director of the institute, for placing this material at my disposal.

but for examination of the differentiation of the neural lobe it had to be slightly modified. Especially the differentiation of the azocarmine had to be slight, since otherwise the colour of the pituicytes will disappear completely. Bodian impregnation for nerve fibres was also extensively used, though I did not succeed as regularly as when dealing with adult material. Especially at their first appearance the fibres are very difficult to impregnate. The method was, however, unailing for impregnation of argyrophilic granules of the glandular cells of the pars distalis, provided that the specimens had been well preserved in solutions not containing chromic acid or its salts. The Gömöry method for reticular fibres proved valuable for demonstrating the development of the neural lobe and was therefore used fairly often. Table 2 gives an idea of the number of embryos treated in the different ways.

Graphic Reconstruction, Illustrations

Graphic reconstructions were made in a number of cases. I then used the method of reconstructing in a plane at right angles to the plane of sections, described by Pantin (1946, p. 50, method 2). In the adult material it was easy to find an axis from which the measurements could be made. When transversal sections were used for the purpose I choose the line along which the median plane cuts the trimmed and plane outer surface of the specimen. In embryos I used the contour of the brain as shown by a median section of an embryo of the same stage. In a few cases I have made a kind of reconstruction in the same plane as the sections (Pantin, l. c. method 1). Thereby I have completed the picture from one section by projecting one or a few preceding or following sections over it. The method used is noted in the legends for each figure.

The *microphotographs* and the *drawings* have been made by the author. The technique used, magnification and filters etc. has been indicated in the legends as far as necessary for a correct understanding of the figures. As the microphotographs are intended to prove the statements in the text they have not been retouched.

Tables

Table 1

The material of adult birds used for the present investigation

After the name of the species¹ the number of preparations of different kind is indicated. The letters used should be interpreted in the following way:

E = entire sphenoid region or entire head preserved.

D = only the pituitary and the hypothalamic region of the brain preserved. (Dissected).

P = only pituitary proper preserved, neurohypophysis usually incomplete.

p = paraffin section.

c = celloidin sections.

t = total preparations, cleared up or dissected.

The figures in brackets indicate the number of specimens used for special methods, and these specimens are included in the preceding figures. The letters in brackets mean:

s = silver impregnation of some slides for nerve fibres (Bodian method).

i = ink injection of the vascular system.

j = starch-cinnobar injection into the arterial system.

The asterisque in some cases indicates that the result was not quite perfect. Either the preservation or the staining was bad, or, the gland had been partly damaged when the animal was shot or when the gland was dissected.

Subclass Ratitae

Order Struthioniformes.

Fam. Struthionidae.

Struthio camelus L. — 2 Dp (2 s*).

Subclass Impennes

Order Sphenisciformes.

Fam. Spheniscidae.

Spheniscus magellanicus (Forster) — 1 Ec, 1 Dp (1 s).

Subclass Carinatae

Order Procellariiformes.

Fam. Diomedidae.

Diomedea epomophora Lesson — 1 Ec.

Diomedea melanophris Temminck — 2 Dp (2 s).

Fam. Procellariidae.

Priocella antarctica (Stephens) — 1 Ec.

Procellaria aequinoctialis L. — 1 Ec, 1 Dp.

Fam. Hydrobatidae.

Oceanites gracilis gracilis (Elliot) — 1 Ep.

Fam. Pelecanoididae.

Pelecanoides magellani (Mathews) — 1 Ec.

¹ The nomenclature for scientific names used in this paper is, for Swedish birds, that of the Swedish check-list (Förteckning över Sveriges fåglar, 1949), for South American birds that of Goodall, Johnson, and Philippi (1946), and for oceanic birds that of Murphy (1936). The system is that used by Berlioz (1950).

Order Pelecaniformes.

Fam. Phalacrocoracidae.

Phalacrocorax magellanicus (Gm.) — 1 Dp (1 s).

Phalacrocorax atriceps King — 1 Ec, 1 Dp (1 s).

Order Ciconiiformes.

Fam. Ardeidae.

Nycticorax nycticorax obscurus Bonap. — 1 Dp (1 s).

Order Anseriformes.

Fam. Anatidae.

Cygnus olor (Gm.) — 1 Dp (in captivity).

Anser anser (L.) — 2 Dp (domestic), 1 Dp (orig. wild, in captivity), (3 s).

Anser indicus (Lath.) — 1 Ec (in captivity).

Branta leucopsis (Bechst.) — 1 Dp (in captivity), (1 s).

Anas p. platyrhynchos L. — 2 Dp (domestic), 3 Dp (wild), (2 s).

Anas clypeata L. — 1 Dp.

Clangula hyemalis (L.) — 1 Dp.

Scoteria m. mollissima (L.) — 1 Dp.

Melanitta n. nigra (L.) — 1 Ec.

Order Lariformes.

Fam. Laridae.

Larus r. ridibundus L. — 3 Dp.

Larus c. canus L. — 4 Dp, 1 Pp (1 Golgi).

Larus a. argentatus Pont. — 1 Ec, 1 Ep, 7 Dp, 5 Pp (2 i, 3 s).

Larus fuscus L. — 1 Dp.

Order Charadriiformes.

Fam. Charadriidae.

Numenius phaeopus hudsonicus Lath. — 1 Ec.

Numenius a. arquata (L.) — 1 Dp.

Capella g. gallinago (L.) — 1 Ec (1 i).

Limnocryptes minimus (Brünn.) — 1 Ec (1 i).

Calidris c. canutus (L.) — 1 Dp.

Tringa t. totanus (L.) — 1 Ec.

Order Galliformes.

Fam. Tetraonidae.

Lyrurus t. tetrix L. — 2 Ec, 3 Dp (2 i*).

Lagopus l. lagopus (L.) — 1 Dp, 1 Dp*.

Fam. Phasianidae.

Phasianus colchicus L. — 2 Dp.

Perdix p. perdix (L.) — 1 Dp.

Gallus gallus (L.), domestic fowl — 3 Dp, 2 Ec (2 i).

Order Columbiformes.

Fam. Columbidae.

Columba p. palumbus L. — 1 Dp.

Columba oe. oenas L. — 1 Pp*.

Columba livia Gm., domestic — 5 Et, 5 Ec, 20 Dp, 4 Pp, 5 for freezing sections
(4 i, 6 J, 11 s, 14 specimens used for other silver methods).

Order Falconiformes.

Fam. Vulturidae.

Coragyps atratus (Vieill.) — 1 Dp (1 s).

Fam. Falconidae.

Milvago chimango temucoensis Selater — 1 Ec.*Accipiter n. nisus* (L.) — 2 Dp.*Accipiter g. gentilis* (L.) — 1 Dp.*Buteo b. buteo* (L.) — 1 Dp.

Order Strigiformes.

Fam. Strigidae.

Asio o. otus (L.) — 1 Dp*.*Strix a. aluco* L. — 1 Ec*, 1 Dp, 2 Pp.

Order Psittaciformes.

Fam. Psittacidae.

Enicognathus leptorhynchus King — 1 Ec.*Agapornis nigrigenis* Sel. — 1 Dp*.

Order Cuculiformes.

Fam. Cuculidae.

Cuculus c. canorus L. — 1 Dp.

Order Piciformes.

Fam. Picidae.

Dendrocopos m. major (L.) — 1 Ec (1 i).*Dendrocopos m. minor* (L.) — 1 Dp.*Picus v. viridis* L. — 1 Ec.

Order Caprimulgiformes.

Fam. Caprimulgidae.

Caprimulgus e. europaeus L. — 1 Ec, 1 Dp.

Order Apodiformes.

Suborder Apodiformes.

Fam. Apodidae.

Apus a. apus (L.) — 1 Ec, 3 Ep, 2 Dp (1 s*).

Suborder Trochiliformes.

Fam. Trochilidae.

Sephanoides sephanoides (Lesson et Garnot) — 2 Ep.

Order Passeriformes.

Suborder Mesomyodes.

Fam. Furnariidae.

Leptastenthura ae. aegithaloides Kittlitz — 1 Ec.

Fam. Tyrannidae.

Elaenia albiceps chilensis Hellmayr — 1 Ec.*Xolmis p. pyrope* Kittlitz — 1 Ec.

Suborder Acromyodes.

Fam. Sylviidae.

Phylloscopus t. trochilus (L.) — 1 Ep.*Sylvia borin* (Bodd.) — 1 Ep.

Fam. Regulidae.

Regulus r. regulus (L.) — 1 Ec, 3 Ep.

Fam. Turdidae.

Turdus pilaris L. — 1 Dp.

Fam. Paridae.

Parus atricapillus borealis Selys-Longch. — 3 Ep.

Fam. Hirundinidae.

Riparia r. riparia (L.) — 1 Ep, 3 Dp.

Fam. Sittidae.

Sitta e. europaea L. — 1 Dp.

Fam. Fringillidae.

Emberiza c. citrinella L. — 3 Ec, 2 Ep* (1 i).

Fam. Ploceidae.

Passer m. montanus (L.) — 2 Ep.

Fam. Corvidae.

Garrulus g. glandarius (L.) — 2 Dp (1 Golgi).*Pica p. pica* (L.) — 1 Et, 4 Ec, 7 Dp (1 s, 1 i, 1 j).*Corvus corone cornix* L. — 7 Dp, 1 Et (1 j).

Table 2

The material of embryos used for the present investigation

The total number of embryos of every size class is indicated and within the brackets I have stated how many of them were stained in azan (a) and impregnated according to Bodian (s). It is to be noted that the slides of the same series were often treated in different ways. The abbreviations mean:

CR = crown-rump length, TL = total length, d = days, a = azan staining, s = silver impregnation according to Bodian.

A. *Gallus gallus*

Less than 3 d: 6, with 0, 12, 17, 23, 23, and 23 pairs of somites resp. (6 a); *3 d:* 3 (1 a, 1 s); *3.5 d:* 1 (1 a); *4 d:* 2 (1 a, 1 s); *4.5 d:* 2 (2 s); *5 d:* 3 (1 a, 2 s); *5.25 d:* 1 (1 s); *6 d:* 5 (1 a, 4 s); *7 d:* 1 (1 a); *8 d:* 2 (2 a, 1 s); *9 d:* 1 (1 a); *9.25 d:* 1 (1 a, 1 s); *10 d:* 2 (2 a, 1 s); *10.5 d:* 1 (1 a, 1 s); *11 d:* 6 (2 a, 4 s); *12 d:* 1 (1 a); *12 d 4 hrs:* 1 (1 a, 1 s); *13 d:* 1 (1 a); *14 d:* 1 (1 a); *15 d:* 2 (2 a, 1 s); *16 d:* 1 (1 a); *17 d:* 1 (1 a); *18 d:* 1 (1 a); *19 d:* 2 (2 a, 1 s); *22 d (1.5 after hatching):* 1 (1 a, 1 s).

B. *Larus ridibundus*

CR 3 mm, 6—7 pairs of somites: 1 (1 a); *CR 7.5 mm:* 1 (1 a); *CR 9.4 mm:* 1 (1 a); *CR 10—15 mm:* 3 (3 a); *CR 16—20 mm:* 5 (4 a); *CR 21—25 mm:* 1 (0 a); *CR 26—30 mm:* 12 (8 a, 3 s); *CR 31—34 mm, TL 50—60 mm:* 4 (3 a, 1 s); *TL 61—70 mm:* 2 (2 a, 1 s); *TL 71—80 mm:* 7 (5 a); *TL 81—90 mm:* 5 (2 a, 1 s); *TL 91—101 mm:* 7 (5 a); *Young TL 13 cm:* 1 (1 a); *Young TL 21—28 cm:* 3 (3 a).

C. *Riparia riparia*

CR 3.5 mm, 8—9 pairs of somites: 1 (1 a); *CR 4.0—5.0 mm,* 20 or more somites: 3 (2 a); *CR 5.1—7.5 mm:* 3 (3 a, 1 s); *CR 7.6—10.0 mm:* 9 (8 a, 4 s); *CR 10.1—12.5 mm:* 10 (4 a, 1 s); *CR 12.6—15 mm:* 5 (3 a, 0 s); *CR 15.1—17.5 mm, TL 26—27 mm:* 3 (2 a); *Hatching stage, CR 19 mm, TL 30 mm:* 4 (1 a, 1 s); *Young TL 43 mm:* 1 (1 a).

CHAPTER II

General Anatomy and Terminology

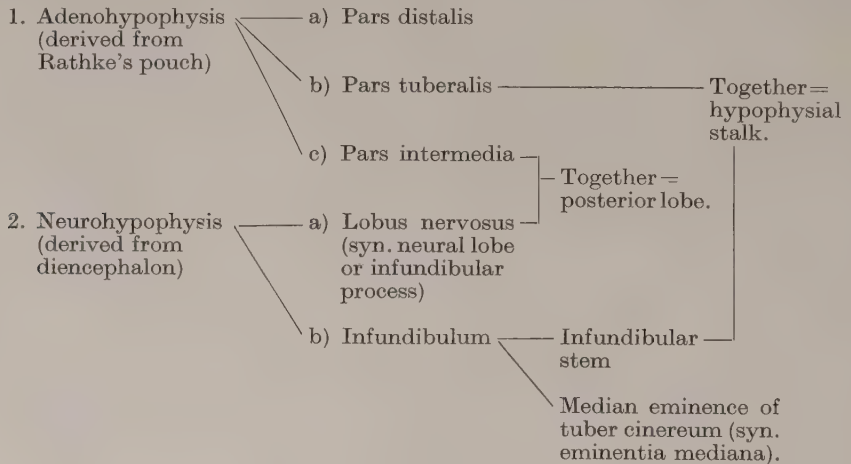
The gross anatomy of the bird pituitary is remarkably uniform throughout the group. The main features of the organ and its subdivisions are shown in fig. 1—5, and apart from size relations and insignificant variations in shape this scheme is applicable to all birds which I have examined. Nor have I been able to find any reliable descriptions in the literature which definitely deviate from this type in essential features. This is strikingly different from the conditions among mammals and reptiles in which the structure of the pituitary is much less homogeneous.

For denoting the orientation of the gland and adjacent structures I have used the following terms: The direction towards the bill is called rostral or anterior, the opposite direction is called caudal or posterior. The direction towards the brain is called dorsal or aboral and the opposite direction is called oral or ventral (compare fig. 1).

The terminology used for the subdivisions of the gland in this paper is that of Rioch, Wislocki and O'Leary (1940), slightly modified according to Green (1947), see table 3.

Table 3

The nomenclature used for the subdivisions of the gland used in the present paper (after Green 1947).



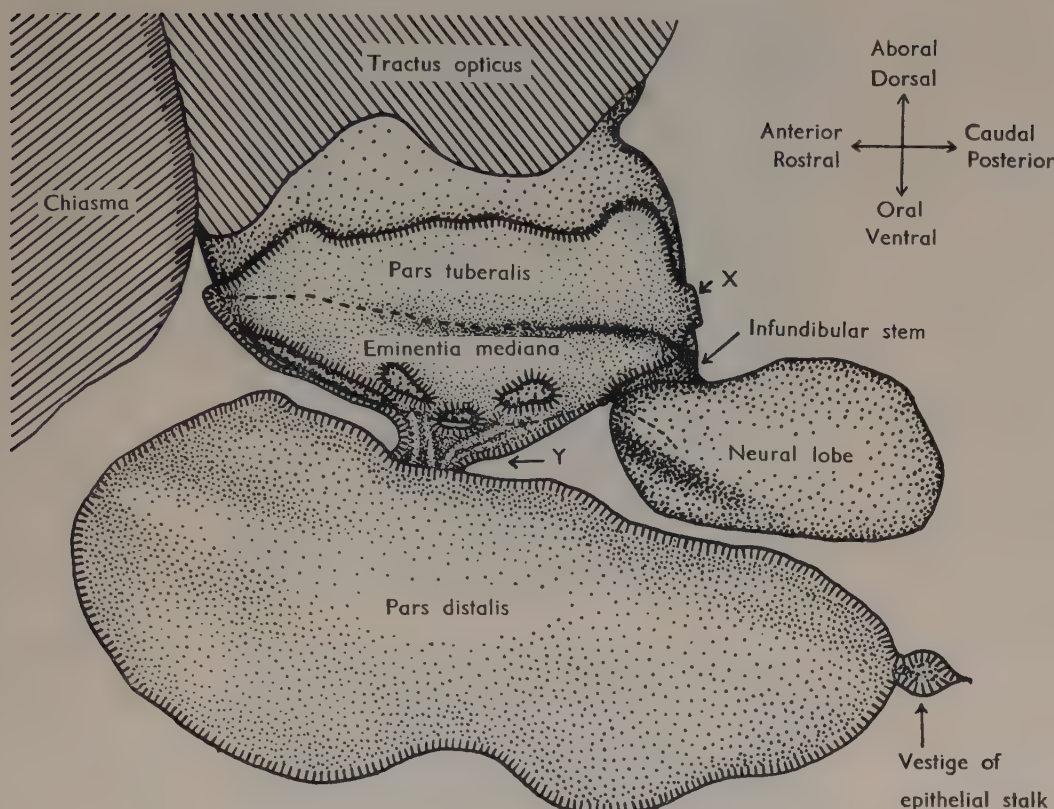


Fig. 1. Pituitary of a goose (*Anser anser*). The dorsal limit of the median eminence is indicated by an interrupted line. X = the junction of pars tuberalis with that of the other side behind the infundibulum. Y = the connection between the pars distalis and the pars tuberalis proper ("portal zone"). — Graphic reconstruction from transversal sections, 21 $\times$.

The bird pituitary, as it is shown in fig. 1, shows some peculiarities which must be observed when using this terminology. The *adenohypophysis* is made up mainly of the pars distalis which is situated ventrally or rostroventrally of the neurohypophysis. An intermedia is not present, a fact which will be discussed further in the following chapters. The pars tuberalis consists of cell strings and lamellae which cover parts of the ventral surface of the diencephalon and usually extends also to the dorsal (caudal) side of the infundibular stem (X in fig. 1), thus forming a collar around the infundibulum. It is connected with the pars distalis by a bundle of strings which originates in the median region of the eminentia mediana and passes to the pars distalis. This division of the pars tuberalis is called "portal zone" in the following because it lodges the portal vessels (Y in fig. 1). The portal zone runs independently of the infundibular stem to the pars distalis (with a few exceptions) and not along it as in many other animals.

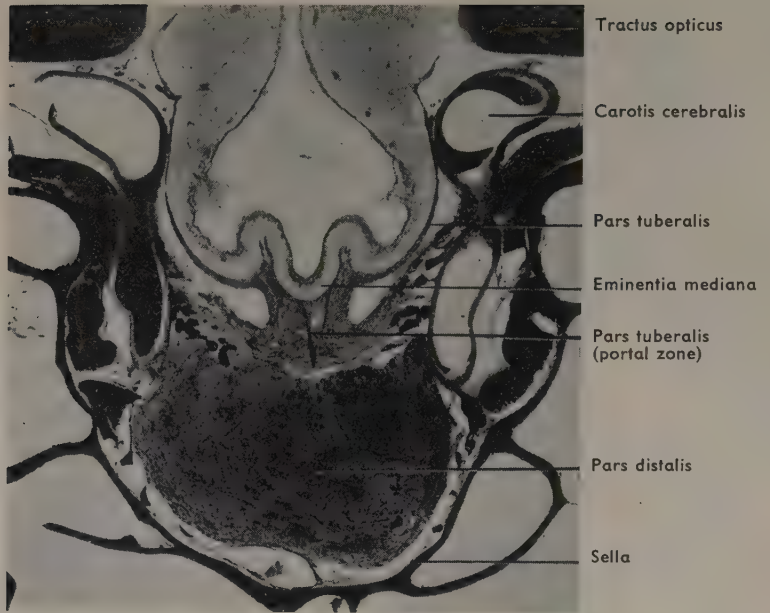


Fig. 2.

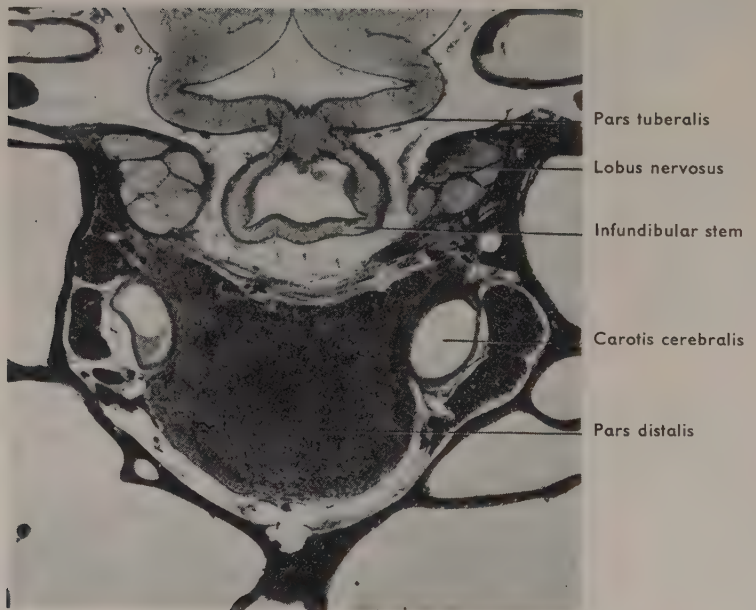


Fig. 3.

The *neurohypophysis* always consists of a distinct neural lobe which is usually heart-shaped or arrow-shaped with the point directed caudally or ventrocaudally. The other two parts, the *eminencia mediana* and the *infundibular stem*, are not distinctly separable in most birds. The former is defined by Green (1947) as "that

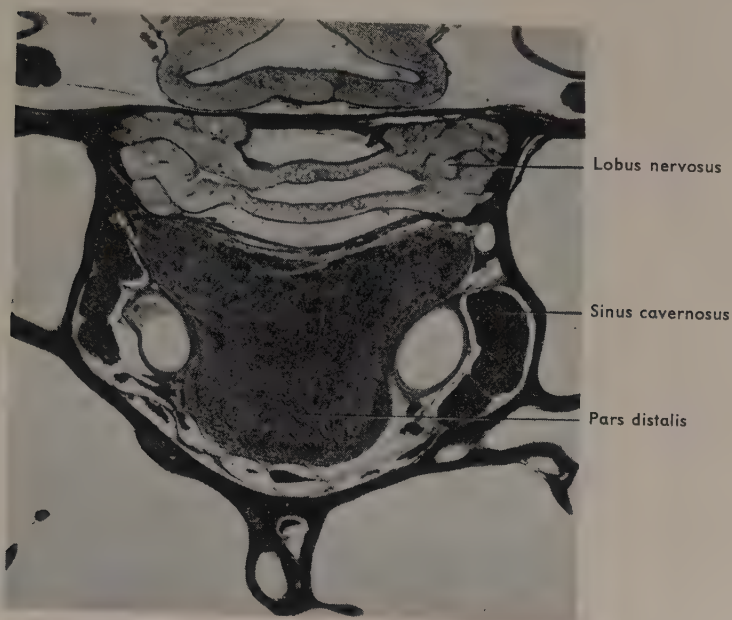


Fig. 4.

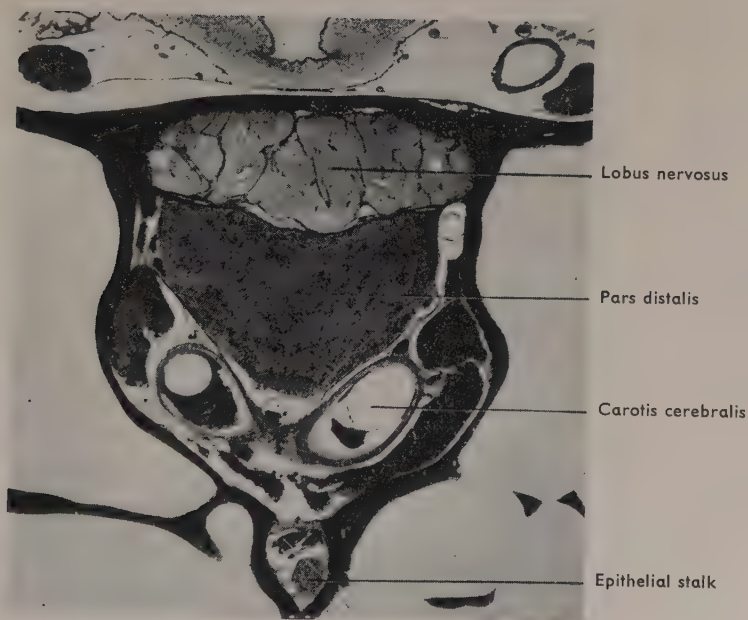


Fig. 5.

Fig. 2—5. Transversal sections through the pituitary region of *Diomedea epomophora*, fig. 2 through the region of the portal zone, fig. 3 through the infundibular stem, fig. 4 through the transitional region between the infundibular stem and the neural lobe, and fig. 5 through the neural lobe. — Bouin, celloidin 60 μ , Mallory's phosphotungstic acid haematoxylin, 11 $\times$.

part of the neurohypophysis which is coextensive with the primary capillary net of the hypophysioportal system of blood vessels, or its equivalent, together with the immediately adjacent parts of the tractus hypophyseus". As will be shown in chapter XIII the capillary net on the surface of the stem is continuous with that of the eminentia, and it is difficult therefore to distinguish the two parts with the help of this definition. There would hardly be any part left to call infundibular stem in birds, but I still retain this term for the narrowed, tubular part of the infundibulum, which carries the neural lobe. For embryological reasons such a separation seems to be justified (chapter VIII). Moreover, the stem is not or only partly covered by the pars tuberalis, the capillary net on its surface is not so dense and the wall often shows some histological differences from the eminentia proper. The differences are, however, only slight, and it is often impossible to find a distinct boundary between these organs.

Among the other terms in table 3 the "posterior lobe" is useless for birds which lack an intermedia.

As to the other terms for embryonic structures, blood vessels, etc. I have referred to the papers used for the nomenclature in the respective chapters, as far as it seemed necessary.

CHAPTER III

The Adult Adenohypophysis

Literature Review

The structure of the adenohypophysis of adult birds has attracted the attention of numerous investigators since the gland was described from avian material for the first time by Malacarne (1782). The essential features in its gross anatomy are therefore now fairly well known for a number of species. The most comprehensive investigation is that of Rahn and Painter (1941) who examined and figured the pituitaries of 18 species of birds. Because of the large material their account is unquestionably the best to be found in the modern literature.

Rahn and Painter state that the adenohypophysis consists of two parts in birds, the pars distalis and the pars tuberalis. Further they have distinguished two histologically different divisions of the pars distalis, which they call cephalic and caudal lobe respectively. They definitely deny the presence of an intermedia. Though some diverging statements have appeared, most modern investigators seem to accept this interpretation of the gross anatomy and it is corroborated also by the present investigation.

The views on the histology are much more diverging. Not even the histological bipartition of the gland mentioned above has been generally accepted, and whereas Rahn and Painter (1941) and Payne (1942, 1944, 1947) distinguish two types of acidophilic cells, the previous authors usually distinguish one. It may be that some discrepancies depend on the general difficulty to examine the histology of the pars distalis in birds — the cells are small, the granulations fine, and the staining reaction often not definite. Another source of divergence is that the authors have used different species for their investigations and further, that all recent histologists — except Rahn and Painter (1941) — have used domesticated birds which are probably more variable than wild ones. The knowledge of the functional significance of the cell types is still little developed in spite of Payne's (1942, 1947), Apor's (1942), Koch's (1942), Schooley's (1937) and Schooley and Riddle's (1936 and 1938) works.

Below I have listed the more important works which describe the structure of the adult adenohypophysis in birds. I have thereby paid special attention to the papers published after 1920, and I have added a few notes on the species examined and on the results. For more complete lists of the older literature see e. g. Stendell (1914) and Bruni (1914).

- Hannover (1844, p. 22) published the first microscopical study of the avian pituitary and stated that it consists of two divisions.
- Stieda (1869, p. 48) studied the pituitary in *Gallus* and observed the arrangement of the glandular cells.
- W. Müller (1871, p. 408) described almost perfectly the gross anatomy of the pituitary in *Columba*. He even saw the portal vessels and the portal zone of the pars tuberalis (called "Arterienzweige" and "Drüsenschläuche" resp., Taf. X: 5).
- Saint-Remy (1892) found chromophilic and chromophobic cells in the pars distalis of *Columba* and regarded them as different functional stages of one cell type.
- Haller (1898, p. 96): *Gallus*, *Emberiza*. The account is somewhat confusing because of Haller's description of a glandular duct which is said to open into the intermeningeal spaces. These statements have never been confirmed.
- Sterzi (1904, p. 106): *Gallus*, *Meleagris*, *Anser*. Found three parts in the pars distalis. The "porzione inferiore", also called "porzione superiore" is the pars distalis, the "prolungamento anteriore" is the pars tuberalis and the "porzione media" may be a mistake or refers to exceptional strings in the same position as an intermedia.
- Fischera (1905): *Gallus*. The pars distalis is said to contain very few acidophils and to consist mainly of basophils and chromophobes.
- Gentes (1907): *Anas*. Original not seen, summary in Gentes (1907a). Believes he has observed a true hypophyseal cleft in the duck.
- Herring (1908, 1913): *Gallus*, *Sturnus*, *Turdus*. Recognized the pars distalis (called anterior lobe) and the pars tuberalis (called pars intermedia), and observed that the latter extends to the caudal side of the infundibular stem in *Gallus*.
- Tilney (1912 and 1914): *Columba* and *Gallus*. In the work of 1914 Tilney clearly realized the different origin of the intermedia (called pars infundibularis) and the pars tuberalis. He uses the term pars infundibularis for an area with mainly chromophobic cells in *Gallus* near the neural lobe.
- Stendell (1913 and 1914): reviews his own and previous works and states that the adeno-hypophysis consists of two parts, the "Hauptlappen" (= pars distalis in the present paper) and the "Zwischenlappen" (= pars tuberalis), but the birds are treated briefly.
- Krause (1922, p. 253): *Columba*. The fig. 109 is quite perfect and shows the pars distalis, the pars tuberalis (called "Zwischenlappen") and even its portal zone and the portal vessels.
- De Beer (1926, p. 29): *Gallus*, *Anas*, *Columba*. Stated the absence of the intermedia and interpreted the pars tuberalis in the same way as modern anatomists.
- Pokorny (1926, p. 320): 10 species, but descriptions only for *Columba*, *Buteo*, *Sylvia*, *Fringilla* and *Apus*. Pokorny regards the pars tuberalis as homologous with the intermedia of other vertebrates and calls it "Zwischenlappen". The cells of the pars distalis are said to be uniform in *Columba* but in *Buteo* Pokorny could recognize basophils and acidophils in the rostral (!) end.
- Desgous (1926 and 1926 a): *Gallus*. Functional relationships between gonads, hypophysis and epiphysis. Lipoid content.
- Collin (1926 and 1926 a): *Anas*, *Gallus*, esp. hypophyseal colloid and cysts.
- Satwornitzkaja and Simnitzky (1932): *Columba*, effect of thyroidectomy.
- Podhradsky (1933, 1935): *Gallus*, weight variations of the pituitary.
- Haller v. Hallerstein (1934, p. 286): review.
- v. Soos (1935): *Meleagris*, *Gallus*, *Anas*, *Columba*, *Anser*, esp. basophils.
- Fésüs (1936): *Anas*, *Anser*. Original not seen, see Zool. Bericht, 43, p. 226.
- Schooley (1937) and Schooley and Riddle (1936 and 1938): *Columba*, esp. histology of the pars distalis. Experimental results.
- Schildmacher (1937): *Turdus merula*. Seasonal changes in the pars distalis.
- Rahn (1939): *Gallus*. Morphology and histology.

- Rost (1939): *Columba*. Morphology and histology.
- Romeis (1940, pp. 242, 277 and 366): *Gallus*, *Columba*. Review of literature.
- Payne (1940, 1942, 1944, 1947): *Gallus*. Histology of the pars distalis. Experimental results.
- Koch (1942) and Apor (1942): *Columba*. Extensive and detailed descriptions of cyclic histological variation in the pars distalis.
- Gorbman (1941): Literature review.
- Rahn and Painter (1941): *Anas*, *Gallus*, *Larus*, *Sterna*, *Columba*, *Chaetura*, *Dryobates*, *Mealanerpus*, *Sayornis*, *Penthestes*, *Baeolophus*, *Mimus*, *Regulus*, *Dendroica*, *Sturnus*, *Richmondena*, *Zonotrichia*, *Melospiza*. Anatomy and histological subdivisions.
- Painter (1942): *Anas*. Morphology and histology.
- Parhon (1946 and 1948): *Gallus* and *Passer*. The basophils are said to be little distinct in *Gallus*, whereas the chromophobes are said to dominate the gland. Parhon could not see any differentiation of the glandular cells in *Passer*.
- Obussier (1948): *Gallus*, *Meleagris*, *Columba*, *Anas crecca* and *A. platyrhynchos*. Gross morphology.
- Bradley and Grahame (1950): *Gallus*. Review.
- Nemec (1950 and 1950 a): *Gallus*, *Canaris*, *Bombycilla*, *Serinus*, *Chrysomitris* (*Carduelis*), *Palaeornis torquata* and *P. alexandri*. Mainly describing the pars tuberalis. In the work 1950 a Nemec describes the connective tissue membranes.
- Benoit (1950): Review.

The physiological aspects on the structure of the avian pituitary have been recently reviewed by Benoit (1950), and by Benoit & coworkers (1944), and this literature has therefore been excluded here.

The Pars distalis

General shape

The outlines of the pars distalis show some variations within the avian class, but as these variations seem to be of slight interest from a comparative and functional point of view they will be briefly treated here. Fig. 6 shows some median sections of the pituitaries of birds belonging to different orders, and the reader will realize at once that none of these types differs in essential features from the scheme given in fig. 1. In fact, all birds which I have examined have a pituitary of this general type, and the variations which will be described below do not concern important characteristics. It should be pointed out in this connection that all the pituitaries shown in fig. 6 were preserved in situ, and that their shape therefore certainly corresponds to the conditions in vivo, though some of them were dissected out before they were cut.

A few outline features may be of systematic significance. Thus for instance, the Galliformes and Anseriformes all have the flattened, elongated type of the pars distalis, shown in fig. 6: G, L, and M. The Passeriformes, on the other hand, are highly variable as is shown by the figures 6: T—Y. One of the types found in the passeriform birds is, however, almost confined to this group, viz. the one of *Xolmis*, *Elaenia* and *Leptastenthura* (fig. 6: T, U). The pars distalis is here highly com-

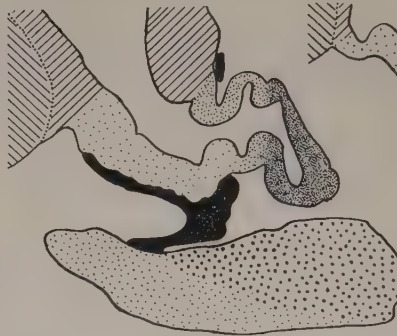
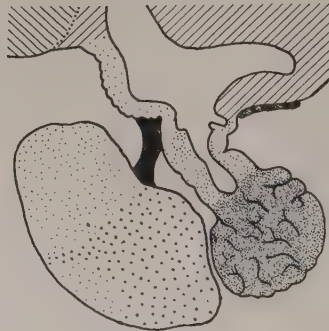
A. *Struthio camelus*B. *Spheniscus magellanicus*C. *Pelecanoides magellani*D. *Diomedea epomophora*E. *Oceanites gracilis*F. *Phalacrocorax atriceps*G. *Cygnus olor* (juv.)H. *Larus argentatus*I. *Calidris canutus*

Fig. 6: A—I.

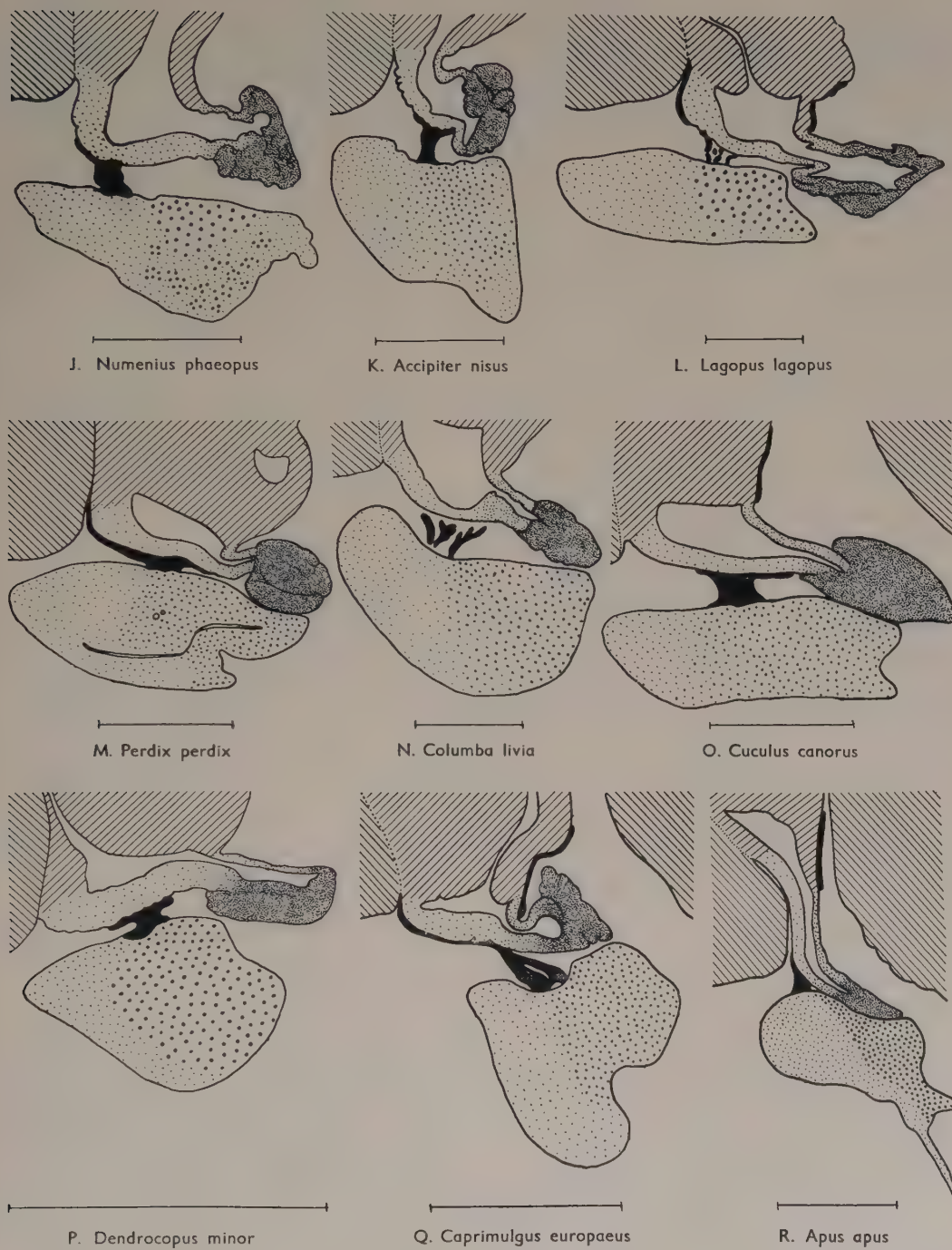


Fig. 6: J—R.

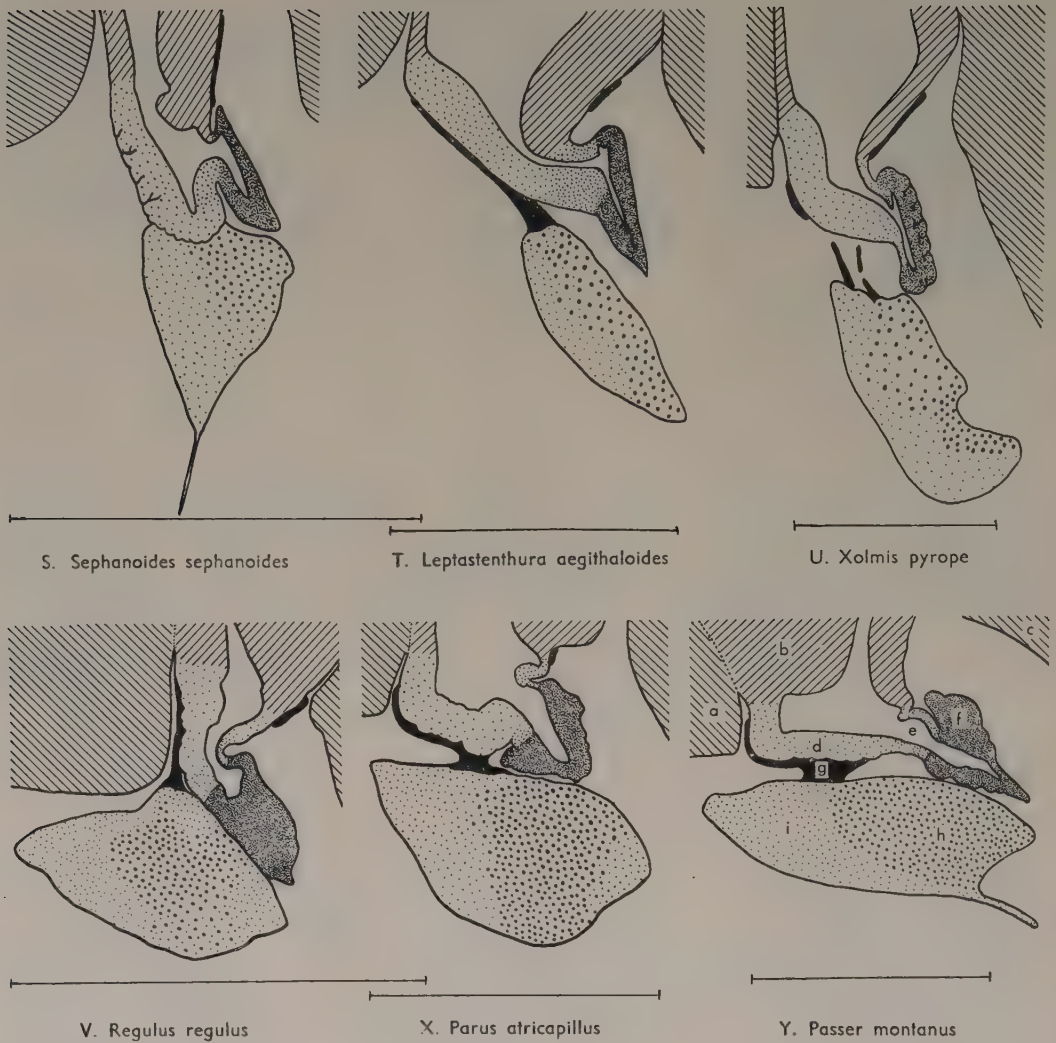


Fig. 6: S—Y.

Fig. 6. Camera lucida drawings of median sections of bird pituitaries, showing the variations in shape. The different parts of the neurohypophysis and of the pars distalis are indicated by different density of the dots and by different size of the dots resp. If, in some cases, the limits are indistinct, this was also the case in the preparation. The pars tuberalis is black. Detailed explanations are given only to fig. Y.

a = chiasma opticum, b = diencephalon, c = medulla oblongata, d = eminentia mediana, e = lumen of infundibular stem, f = neural lobe, g = pars tuberalis, h = caudal lobe of the pars distalis (distribution of A_1 -cells), i = cephalic lobe of the pars distalis.

Figures A, B, D, G, H, I, K, L, M, N, P, and Q from glands fixed in situ but dissected out before embedded in paraffin, figures E, R, S, V, X, and Y from paraffin sections of glands in situ, and C, F, J, O, T, and U from celloidin sections of glands in situ. One millimeter is projected under each figure to show the scale.

pressed rostrocaudally as seen in a sagittal section. That this interpretation of the peculiar shape is right, will be clear from the histological features, which are indicated by the dots in the figures (cf. also p. 55). Rahn and Painter (1941) observed this type of pituitary in another tyrannid (*Sayornis phoebe*). It may be that this shape is characteristic of the primitive passeriform birds of the suborder Mesomyodes (Clamatores). The only bird outside this suborder with a similar type of pituitary is the swift (*Apus*, fig. 6 R) and some tendency to develop in this direction is found in the humming-bird *Sephanoides* (fig. 6: S). It is of interest in this connection that Lowe (1939), after careful anatomical investigations, found that the swifts and hummingbirds are closely related to the passeriform birds and that they can even be included in the latter order as suborders.

Within the same species, however, the individuals seem to be more constant as to the shape of the pars distalis. Thus, for instance, all the six swifts (*Apus*) in my collection have a pars distalis of the peculiar appearance shown in fig. 6: R, and the still more deviating outlines of the adenohypophysis shown in fig. 6: S are present in both humming-birds of the species *Sephanoides sephanoides* which I have examined.

In domesticated duck and fowl there is considerable individual variation in the shape of the pituitary, as shown by Obussier (1948). According to his investigations, however, the variation of the pituitary is greater in domesticated animals than in wild ones, and my general impression is that this is true also for birds. This was also premised by Obussier though his material of birds did not suffice for definite conclusions.

Epithelial Stalk

The pars distalis often presents a conical or thread-like projection from its ventral or caudal surface. This process consists of epithelial cells and is the vestige of the connection between the adenohypophysis and its point of origin in the oral epithelium. Atwell (1939) who called this string "epithelial stalk" stated that it has partly obliterated already in older embryos of *Gallus*, but Obussier (1948) believes that it may sometimes persist also in the adult. This epithelial stalk is of some interest, for it will help us to understand how Rathke's pouch was transformed when the glandular body developed. The epithelial stalk can be studied only in section series of the entire sphenoid region because it is invariably destroyed if the gland is dissected out. I have found considerable remnants of the epithelial stalk in a number of birds, but only in one case did I find a continuous string from the gland to the oral epithelium, viz. in one specimen of *Apus apus*. The other two birds of this species which were sectioned in situ had distinct though discontinuous epithelial stalks, attached to the gland as shown in fig. 6: R.

The examination of the skulls of *Corvus corone cornix* and *Pica pica* revealed a constant and open canalis craniopharyngeus through the sphenoid bone, and it is probable that this channel often contains a continuous epithelial stalk. In fact,

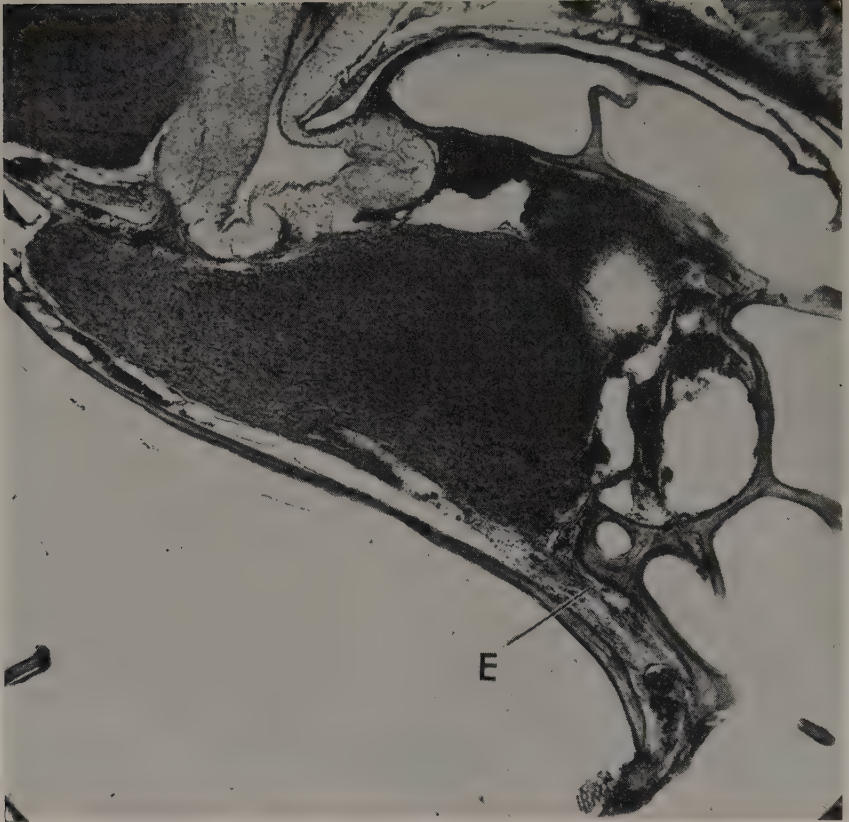


Fig. 7. Median section of the pituitary of *Pica pica*. The gland was cut in situ so that the epithelial stalk (E) and the canalis craniopharyngeus are visible. The oral surface was trimmed away. — Bouin, celloidin 60 μ , phosphotungstic acid haematoxylin of Mallory. 32 $\times$.

the upper part of this channel contains a distinct stalk in the four specimens of *Pica* cut in situ (fig. 7). Yet I could not state whether the string is continuous throughout or not in three of the specimens since the oral surface had been trimmed away, and in the fourth the stalk was not completely continuous. Considerable parts of the epithelial stalk were also observed in the two *Sephanoides* and in one adult specimen each of *Spheniscus magellanicus*, *Diomedea epomophora* (fig. 5), *Anser indicus*, *Melanitta nigra*, *Enicognathus leptorhynchus*, *Xolmis pyrope*, *Emberiza citrinella*, and in one newly hatched chick (*Gallus*).

Usually the epithelial stalk is attached to the ventral or caudoventral surface of the pars distalis, but in the Anseriformes (*Melanitta*, *Anser indicus*, *Anser anser*) it starts from the posterior end of the gland (fig. 1). The latter position was also found in *Anas platyrhynchos* by Obussier, who pointed out that this indicates a peculiar mode of torsion of Rathke's pouch during the embryonic development. In most species the epithelial stalk points caudo-ventrally from its origin in the

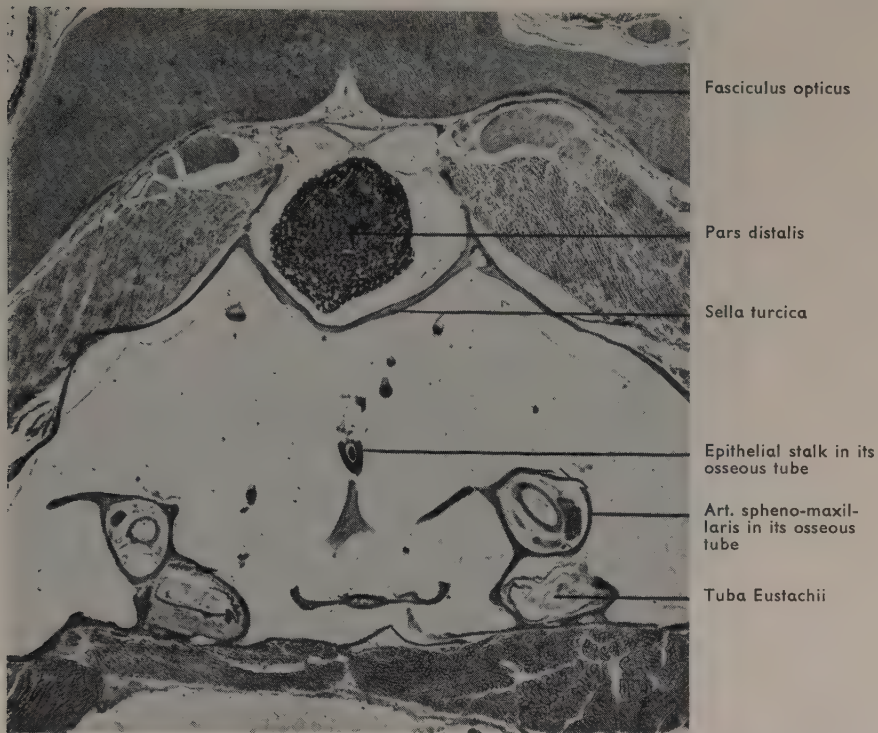


Fig. 8. Transversal section through the sphenoid region of a swift (*Apus apus*, ♀ ad.), to show the passage of the epithelial stalk through the air caverns of the sphenoid. — Bouin, paraffin, 12 μ , haematoxylin of Ehrlich + eosin. 24 $\times$.

gland and then suddenly turns into a rostro-ventral direction. This feature is explained by dislocations during the embryonic development.

The epithelium of the stalk consists of strings and lumps of cells which often enclose large cysts. The cells themselves have a hyaline plasm and I have found no signs of a glandular activity there.

When the epithelial stalk is present — completely or partly — it lodges in the osseous canalis cranio-pharyngeus, which runs like a more or less continuous tube through the extensive air-chambers of the sphenoid (fig. 8). In birds in which the epithelial stalk is fragmentary, I have found the most distinct remnants of it near the pars distalis on the one hand and near the oral surface on the other hand, whereas it has obliterated in the central parts of the sphenoid. It is continuous with the oral epithelium in the groove from which the two tubae Eustachii start, the so-called anthrum tubarum (Pinaroli 1926).

It might be tempting to regard the persistence of the stalk as a primitive feature from a phyletic point of view, since a gland with a complete stalk corresponds approximately to the 9-day stage in a chick embryo. This is, however, certainly not justifiable. As mentioned above, it probably occurs very regularly in the Corvids

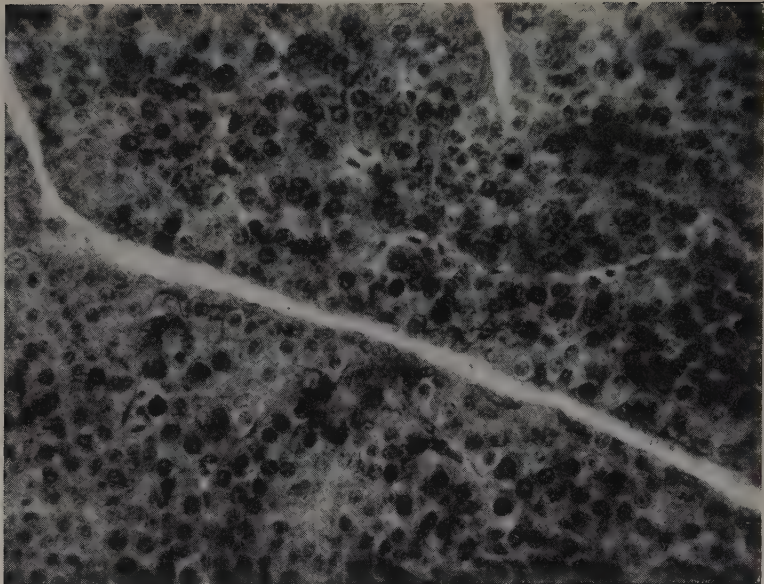


Fig. 9. Residual cleft in the pars distalis of a partridge (*Perdix perdix*). The same gland as fig. 6: M and fig. 76: C and D. — Susa, paraffin 6 μ , azan. 450 $\times$.

which in many respects are the most advanced of all birds, whereas it is usually absent in the Galliformes which have retained many primitive features (cf. Portmann, 1935). Apart from the Corvids in which the stalk may be fairly constant, it is certainly a variable structure. I found, for instance, a distinct though discontinuous epithelial stalk in one of the *Emberiza* specimens whereas the other four have no or only indistinct rudiments. Rost (1939) found an epithelial stalk in one 2-day pigeon and in two adult specimens whereas it is absent in most pigeons after hatching. Though the epithelial stalk gives some interesting indications as to the development of the gland it is thus largely independent of systematic categories and is, in most cases at least, an accidental structure.

Residual Cleft

The adenohypophysis of adult birds is usually compact and the lumen of Rathke's pouch (residual cleft, hypophysial cavity) disappears at an early stage. Also this embryonic feature may, however, sometimes be preserved in the adult. I have found distinct remnants of this lumen in two adult specimens of *Columba livia*, one adult *Columba oenas*, one adult *Perdix perdix* and in young specimens of *Larus ridibundus* and *L. argentatus* (fig. 6: M, fig. 9). The lumen itself is very narrow in the sections and may have been still smaller in the living tissue, but the character of a residual cleft is apparent from the regular arrangement of the epithelial cells which border it. In the specimen of *Perdix* almost all the embryonic lumina in the gland are preserved though only part of them are visible in the

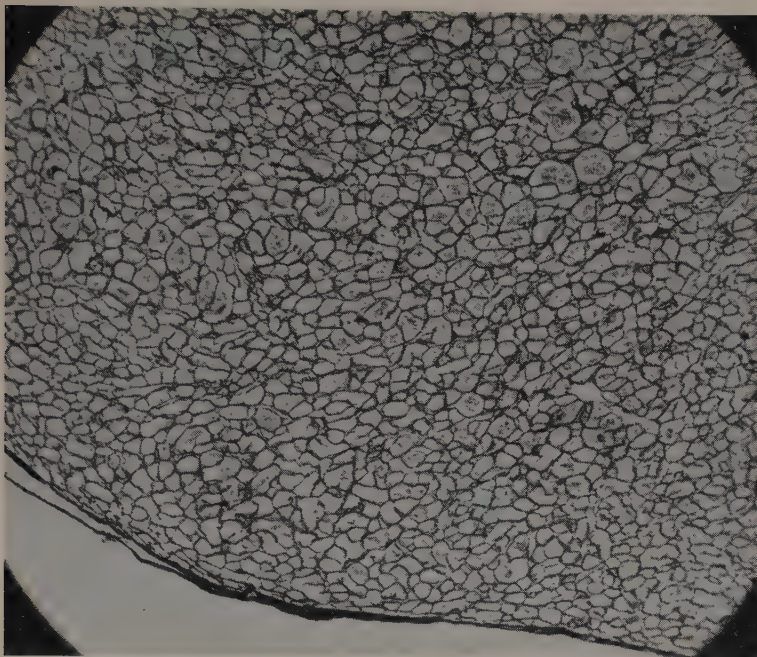


Fig. 10. Transversal section through the pars distalis of a goose pituitary (*Anser anser*), showing the reticular membranes which surround all cell cords and vessels. — Alcohol-formalin-acetic acid, paraffin 8 μ , silver impregnation according to Gömöry. 90 $\times$.

median section drawn in fig. 6:M. Also some of the cysts which occur in bird pituitaries are undoubtedly remnants of the original lumen of the gland. As this question is fairly complicated it will be dealt with separately (chapter VII).

Arrangement of Glandular Cells

The glandular cells in the avian pars distalis are arranged in strings and lamellae which branch and anastomose (fig. 10). The strings are surrounded and partly separated from each other by a dense plexus of wide blood sinusoids. The strings are usually so narrow as to allow practically all cells to reach the external surface, that is, they measure about 2—3 cell diametres. Although nuclei are often seen in the centre of the cell strings and the cell bodies do not seem to reach the surface in that particular section they may do so in another section. The small chromophobes in the centre of the strings seem, however, to lack contact with the surface in many cases.

Non-epithelial Structures

The cell cords are surrounded by a delicate membrane which appears as a distinct blue line in the azan preparations. If the slide is impregnated with silver according to Gömöry (R § 1533) this membrane turns out to be a dense net of argyrophilic

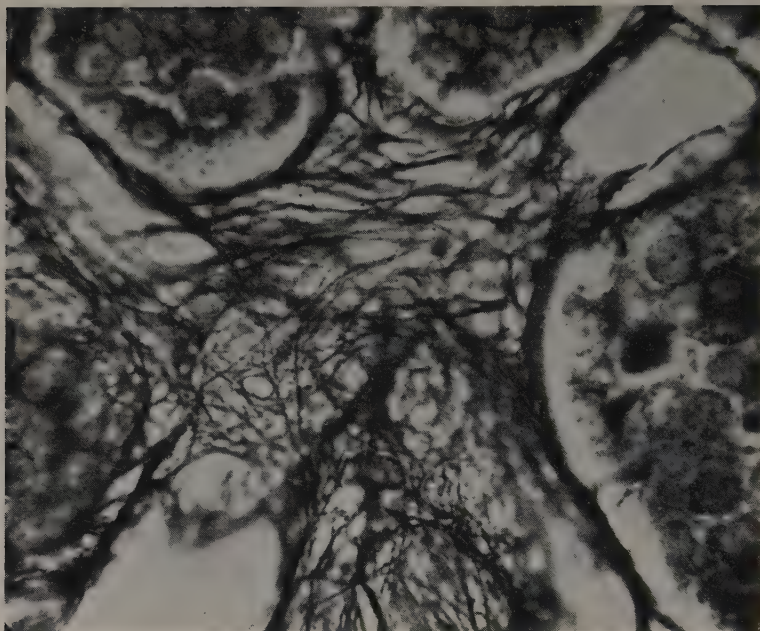


Fig. 11. Reticular membranes in the pars distalis of a pigeon pituitary (*Columba livia*), made visible by silver impregnation. The membranes were here cut partly tangentially. The coarse fibres in the upper part of the picture belong to perivascular membranes, the finer fibres to the left below belong to basal membranes of cell cords. — Fixed by injection of Bouin into the carotid system, paraffin 10 μ , silver impregnation according to Gömöry. 1280 $\times$.

fibres which cross each other in all directions (fig. 11). According to Romeis (1940, p. 228 ff.) the cell strings in the mammalian pars distalis are separated from the blood in the vessels by two such membranes. One is the basal membrane of the glandular cells, the other is the membrane supporting the endothelium of the blood sinusoids. In fact, this can also be observed in birds in such places where one blood sinusoid and two epithelial cords meet to form a triangle between them. The two membranes are here separate from each other and may be observed in azan-stained as well as in Gömöry-preparations (fig. 12). In most places, however, the two membranes seem to have fused completely and it has not been possible to decide whether the resulting wall is still double. The narrow spaces between the basal membrane of the glandular cells and the one around the vessels are traversed by argyrophilic fibres similar to those in the membranes proper. Collageneous fibres usually play an inconsiderable role in the avian pars distalis, and in small species at least there are only few and delicate fibres with less distinct collageneous reaction. In medium-sized birds as gulls, crows etc. there are also fairly coarse fibres near the pars tuberalis along the main branches of the portal vessels, and in some large species, e. g. *Struthio*, *Diomedea*, *Anser*, *Spheniscus*, they are scattered

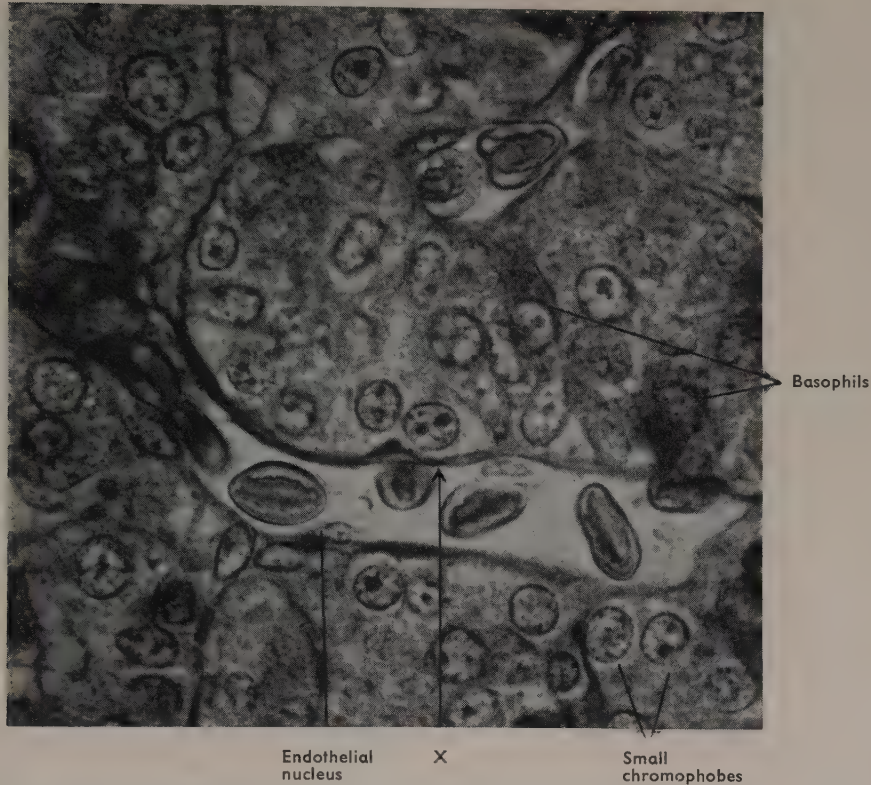


Fig. 12. Blood sinusoid with surrounding epithelial strings in the cephalic lobe of a mag-pie (*Pica pica*, ♀ ad.). At X two membranes are distinct between the epithelial cells and the lumen of the vessel: the basal membrane of the epithelium and the perivascular membrane. The dark epithelial cells are basophils, the light ones are A₂-cells with the exception of a few small chromophobes. — Bouin, paraffin 3 μ , azan. Red filter. 1500 $\times$.

all over the gland. Along the periphery of the gland all these fibres are continuous with the surrounding capsule, which mainly consists of collageneous connective tissue.

The Colloid Acini

More than 60 % of the adult pituitaries which I have examined contain chromophilic colloid in the shape of sphaerules or droplets in the centre of the cell strings. In most cases the amount of colloid is moderate, but in 25 out of 126 adult glands available for detailed examination the acini are so numerous that the sections almost give the impression of a thyroid. In young birds which have not attained their definite size such acini are not present or are very few and undeveloped. Thus the acini do not seem to occur in a high frequency until the bird is full-grown, or nearly so and this is supported by previous observations. Payne (1942, p. 107) found the first acini in white leghorn during their third year of life.

Payne regards the colloid acini as an indication of ageing in the fowl, and my observations partly confirm this view for birds in general, at least if the concept "ageing" is used in a wide sense for all phenomena occurring with increasing age of the animal. In some birds, however, the acini occur before sexual maturity is attained. I have preparations of young herring gulls (*Larus argentatus*) from their second and third year of life, in which the colloid acini are abundant. Some colloid is even present in a specimen aged approximately 14 months. Further it should be mentioned that not all of the adult bird pituitaries contain colloid, and there is a considerable variation within the same species, which may be cyclic, individual, or both. Neither does the amount of colloid necessarily increase with age. In a goose (*Anser anser*, originally wild), which had been kept in the park ponds of the town of Lund for at least 25 years the amount of colloid was insignificant. It was even difficult to find any acini at all in this specimen.

The colloid droplets stain blue in azan, sometimes with a red centre. They are situated in the centre of the cell strings and are bordered by the glandular cells without separating membranes. The surrounding cells are of different kinds. In an adult *Larus canus* in my collection there are, for instance, exclusively dark acidophils around the colloid sphaerules in the caudal part of the gland, or the strings contain in addition also a few chromophobic cells. In the rostral end of the same gland the dark acidophils (A_1 -cells) are absent and the colloid sphaerules are surrounded by basophils and by cells of a more or less chromophobic appearance. In other pituitaries the A_2 -cells, the less stainable kind of acidophils, predominate in the rostral part of the pars distalis. As also the colloid acini tend to be more common in this part of the gland it is natural that the A_2 -cell play a proportionally considerable rôle in the surroundings of most colloid droplets. Thus I can not agree with Payne (1942) when he states that the cells around the colloid droplets "are not granular and do not have the appearance of either actively secreting basophils or acidophils", but I admit that the nearest zone of plasm in the surrounding cells is often less granular (fig. 13).

As to the origin of the colloid acini two questions arise: First, which kind of cells gives rise to the colloid, and secondly, how is the colloid secreted?

As to the first question Romeis (1940, p. 156 ff.) stated for mammals that colloid can be produced in any kind of glandular cells in the pars distalis. Voigt (1939) is, however, apparently of the opinion that this function is restricted to the basophils in the fowl. Accepting Voigt's opinion it is difficult to explain the presence of colloid in the compact area with acidophils in the gull mentioned above. Besides, colloid acini of the same kind are produced also in the pars tuberalis where no chromophilic cells at all are present. These preliminary considerations indicate that production of the acinar colloid is not restricted to the basophils, and this indication is strongly supported below. In fact, the varying histological nature of the surrounding cells makes it probable that the production of colloid is not restricted to any particular cell type.

The colloid acini in mammals probably arise in two different ways (Romeis

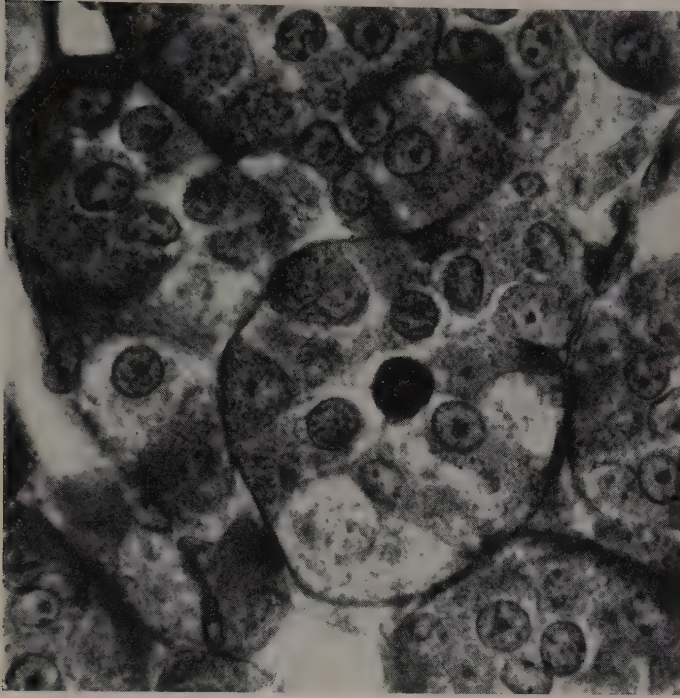


Fig. 13. *Columba livia*, domestic. Colloid acinus in the caudal lobe of the pars distalis. The colloid droplet is mainly surrounded by distinctly granular, dark acidophils (A_1 -cells). — Bouin, injected from the carotis, paraffin 5μ , azan. Yellow filter. $1200\times$.

1940, p. 156 ff.): a) by secretion of colloidal matter from the cells of a cord and accumulation of this intercellular colloid in the centre of the cord (merocrine secretion), and b) by accumulation of such substances in a centrally situated cell which disintegrates and forms the initial colloid droplet (holocrine secretion). Voigt (1939), working with fowl, is convinced of the presence of both these kinds of secretion in the basophils of the fowl, and he even speaks of "holocrine" and "merocrine basophils" as two different kinds of cells. He seems to regard the "merocrine basophils" as responsible for the production of the colloid droplets, although he admits that also holocrine cells may play a rôle. Voigt's opinion that these two kinds of secretion represent the normal mode of activity of the basophilic cells does not seem to be sufficiently supported by facts, but his descriptions of the production of colloid are corroborated by my investigations. Collin (1926) working with pituitaries of fowl and goose concluded that colloid is formed by basophilic disintegration of cells, that is, by holocrine secretion. Thereby the cells could degenerate one by one ("fonte élémentaire") or together in large aggregates ("fonte massive"). As mentioned above the formation of the colloid is not restricted to the basophils and thus probably has nothing to do with the normal function of the basophilic cells. Otherwise my observations on the production of colloid are in

good agreement with Voigt's statements and the secretion seems to occur in a way very similar to that described for mammals. That is, two different processes contribute to the same result:

On the one hand, the centres of the cell cords are sometimes occupied by cells packed with blue-staining masses and in different stages of disintegration (fig. 14—15). This was most distinctly observed in some of my specimens of *Pica*, but also e.g. in one of the *Diomedea melanophris*. Such observations are, however, fairly few. It is necessary to note here that real nuclei were present in the disintegrating cells, often with the nuclear structure fairly intact. The diffuse red centres which can be observed in many colloid droplets are not of importance in this connection — similar centres can often be seen in the colloid sphaerules of the thyroid, and need not have anything to do with nuclei.

In the specimens of *Pica* it was possible to find the entire series of transitional stages from cells packed with blue-staining masses to the homogeneous colloid droplets in the acini. My material, however, did not allow me to decide definitely from which kind of cells these holocrine cells arise. Colloid droplets of variable size and frequency have, however, been observed in several light-staining cells, mainly A₂-cells and chromophobes.

The holocrine secretion, discussed above, is not very frequent, since "nucleated" colloid droplets have been observed only a few times. The merocrine mode of secretion seems to be more common. The preparations show that the colloid appears in young herring gulls (*Larus argentatus*) during their second year of life. In specimens of this age I have never seen any "nucleated" colloid droplets which could indicate a holocrine origin. On the contrary, many colloid droplets are smaller than a cell body, sometimes even smaller than a nucleus, and can therefore hardly have been formed by disintegration of a cell, packed with colloid. It is very probable therefore, that these droplets have a merocrine origin. In one specimen of *Pica*, shot in October, it was even possible to trace the details of this merocrine secretion, partly due to the thinness of the sections (1—3 μ). In this bird there is apparently a very intensive development of colloid acini, probably depending on some exceptional physiological conditions, and abundant material is thus available for study. Several disintegrating cells packed with colloid are present. In addition to these holocrine cells there is also a merocrine formation of colloid, and this is much more frequent. Small colloid droplets can often be observed in the parts of the cells which border the centre of the cell strings. In other places these droplets can be seen in the small cleft which usually occupies the centre of the strings, and in still other cases the droplets have fused and formed extracellular colloid droplets of different size. It can also be seen in which way the colloid droplets penetrate the cellular membrane and become extracellular. Often the droplets have fused with the extracellular colloid mass, but they still reach far into the plasm of the surrounding cells. It is evident that the cellular membrane simply bursts when the colloid vesicle is emptied into the central space (cf. fig. 16).

It may be mentioned that such colloid droplets have been found mainly in the

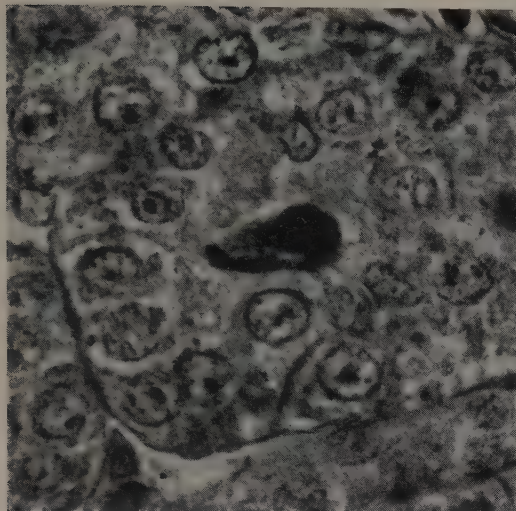


Fig. 14.

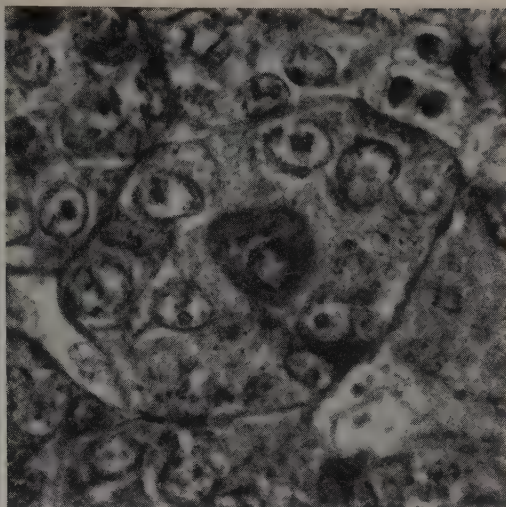


Fig. 15.

Fig. 14—15. Holocrine secretion of colloid in the pars distalis of a mag-pie (*Pica pica*, ad., 19. X.). In fig. 14 the dark disintegrating cell in the centre of the string is surrounded by A_2 -cells, in fig. 15 there is also a dark basophil. The nucleus of the disintegrating cell is visible in both cases. — Helly, paraffin 3μ , azan. Red filter. $1700\times$.

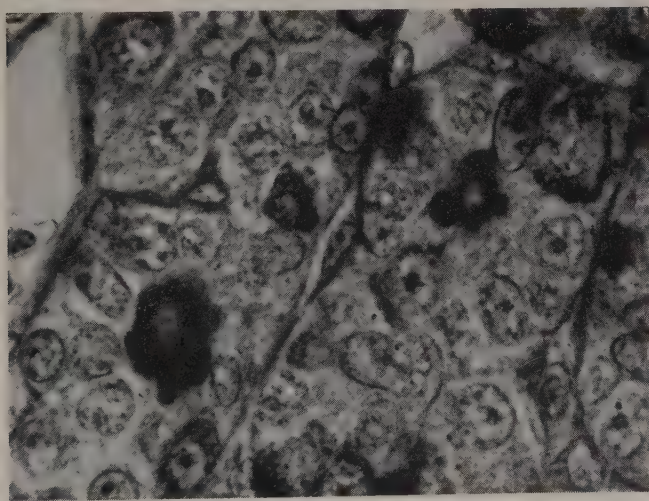


Fig. 16. Merocrine secretion of colloid in the pars distalis of a mag-pie (*Pica pica*). To the right some colloid droplets have been formed near the central space of a cell string but are still largely intracellular and have not fused completely. To the left an older droplet which receives contributions from the surrounding cells. — The same section as in fig. 14—15. Red filter. $1700\times$.

parts of the cell which are opposite to the basal membrane. It is often easy to state the nature of the colloid-producing cell, because the other parts of it have an entirely normal appearance. In all cases in which a certain identification could be made, the cell producing colloid was a light acidophil (A_2 -cell) or a chromophobe. It should be pointed out, however, that the colloid is difficult to detect in the coarse-granulated and heavily stained A_1 -cells and basophils.

Finally, it can be concluded that formation of acinar colloid is distinctly different from common basophilic function, because it may occur also in light acidophilic cells and in chromophobes. Voigt (1939) says that the colloid may arrive also in the vessels, either by merocrine or by holocrine secretion, and need not necessarily be deposited in the strings. My material does not contradict this statement. It seems perfectly clear, however, that Voigt's descriptions refer only to the secretion of acinar colloid and not to the function of normal basophilic cells. I have not observed any holocrine or merocrine secretion of stainable substances in typical basophils. It should also be remembered that formation of acinar colloid takes place in the pars tuberalis and in some parts of the pars distalis where basophils are absent.

The Glandular Cells

The glandular cells of the pars distalis have caused considerable difficulties and they have been dealt with in many papers during the last three decades (p. 27—29). Most authors agree in distinguishing three different types of cells — acidophils, basophils and chromophobes. These terms have not always been used in the same sense, and the light-staining acidophils of the rostral portion of the gland have certainly sometimes been referred to as chromophobes and sometimes as acidophils. Some recent authors distinguish four types of cells, separating the light-staining acidophils from the dark-staining ones. I use this latter terminology, following Rahn (1939), Rahn and Painter (1941), Painter (1942) and Payne (1942), and classify the cells into basophils, chromophobes, dark-staining acidophils (A_1 -cells) and light-staining acidophils (A_2 -cells). I wish to point out, however, that this system is accepted because it is useful for descriptive purposes. Whether these cell types can be regarded also as functionally different will be discussed below.

The following descriptions are mainly based on material fixed in Bouin or Susa and stained in azan. In order to compare the influence of different fixation methods I preserved a series of pituitaries of *Corvus corone* and *Pica pica* in different ways. The birds were shot in October—December and the following fixations were used: Bouin, Susa, Helly, Dawson-Friedgood (1938), and alcohol-formalin-acetic acid. 1—4 μ paraffin sections were prepared and stained in azan. Apart from shrinkage caused by the alcohol-formalin-acetic acid the distinction of the cell types was not very different in the series. The series was valuable also because it shows that the exceptional histological features observed in crows and magpies from this season are real and do not depend on inadequate fixatives.

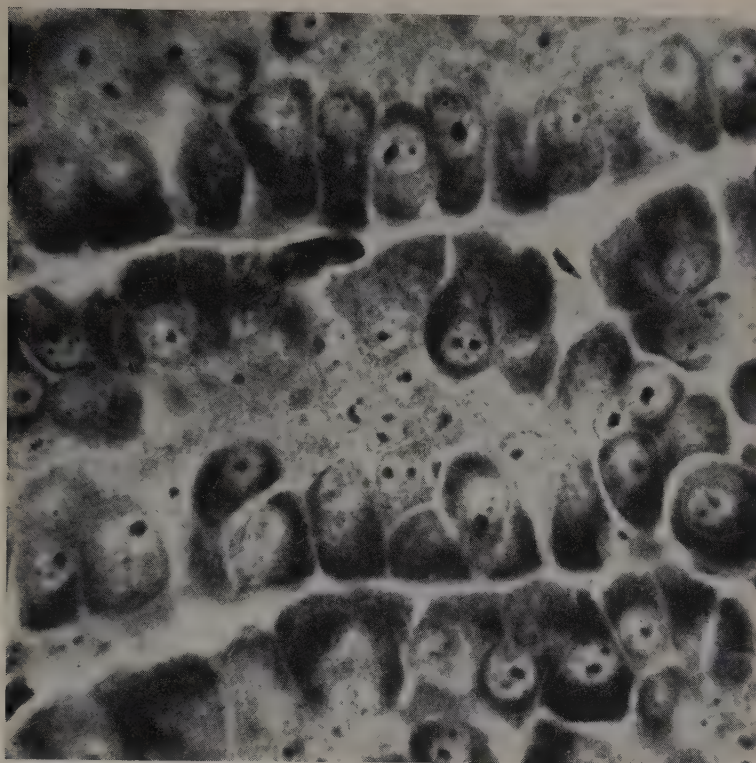


Fig. 17. Appearance of the A₁-cells in a young gull (*Larus ridibundus*) after staining in iron haematoxylin. Detail of the section shown in fig. 26. 1300 X.

The Bodian technique also proved valuable for the examination of the cell types of the pars distalis, because some cells become specifically impregnated. The great advantage of this method is the perfect clarity and distinction of the impregnation, which makes the interpretation of the result quite objective. It is thus a valuable complement to the azan staining which is often difficult to interpret with certainty. It is, however, not easy to compare the result of Bodian staining with the effect of common azan staining. I therefore worked out the following method which makes it possible to compare the results of the two methods in one and the same section:

The sections (not more than 4μ) are first impregnated according to Bodian, gilded, and mounted in balsam. Some typical areas are photographed. The cover-glasses are removed in toluene and the slide is run through graded alcohols to water. The gold in the sections is removed by treating the slide with a cyanide solution (1—2 % potassium cyanide is neutralized with weak acetic acid until phenolftalein is decolorized. Caution — HCN is easily liberated!). The sections must remain in this solution for about one hour and the effect of the treatment is controlled under the microscope before it is stopped. After washing the slide in water azan staining can be made with the same result as if the section had not been impregnated. The photographed areas are now re-examined and photographed again if necessary, and it is now possible to state with certainty which cells in the azan preparations contained the argyrophilic granules (fig. 19—20, 22—23).

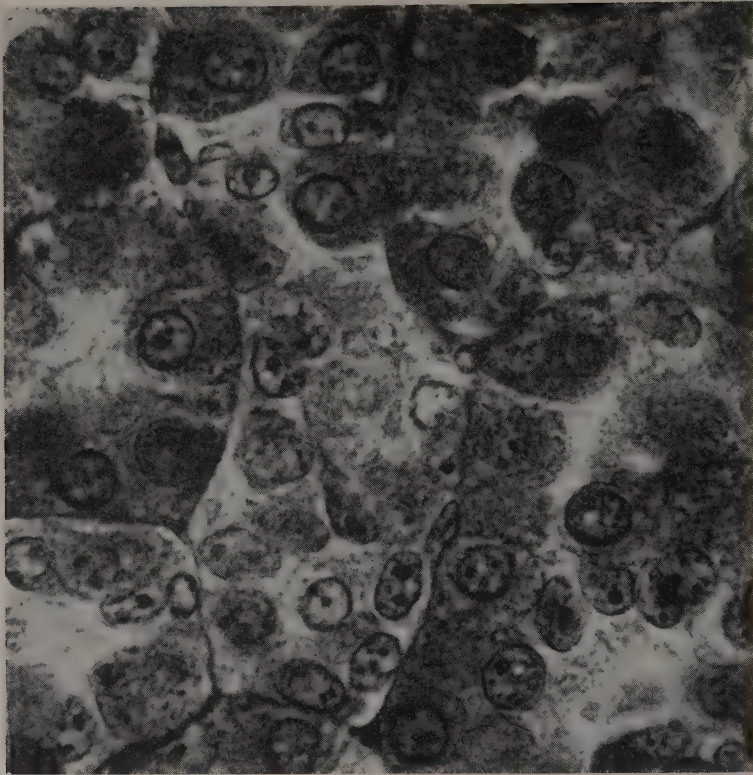


Fig. 18. *Asio otus* (♀ juv., plumage complete, 23.V.). Part of the caudal lobe of the pars distalis. Most cells are A_1 -cells with abundant plasm and coarse granules. In the centre and below there are some chromophobes with light and sometimes very little plasm. — Bouin, paraffin 4μ , azan. Yellow filter. $1500\times$.

This method has been tried only on the abovementioned series of crows and magpies. However, a Bodian impregnation of thin sections directly followed by azan staining will also be useful for comparing the two methods, and this possibility has been widely used.

The granules which become visible after Bodian impregnation are called Ag-granules or argyrophilic granules in the following, and the cells, containing these granules are called Ag-cells. This does not mean that they constitute a special kind of cells comparable with basophils etc. It only means that they contain granules which reduce silver in the Bodian process.

The following description of the cells of the pars distalis holds generally for the birds which I have examined:

1. *The A_1 -cells* (Payne) or *dark-staining acidophils* (Rahn and Painter). These cells are easily identified in most cases by their coarse granules which are stained dark red by the azocarmine. The granules also become distinctly black after staining in Heidenhain's iron haematoxylin. They are impregnated by the Bodian

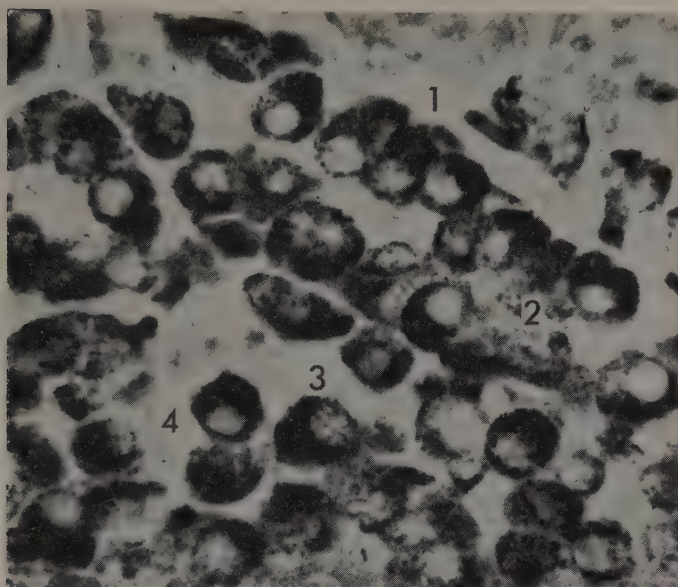


Fig. 19. *Pica pica* (ad., 19. X.). The appearance of the caudal lobe after Bodian impregnation. — Helly, paraffin 3 μ , Bodian. 1200 $\times$.

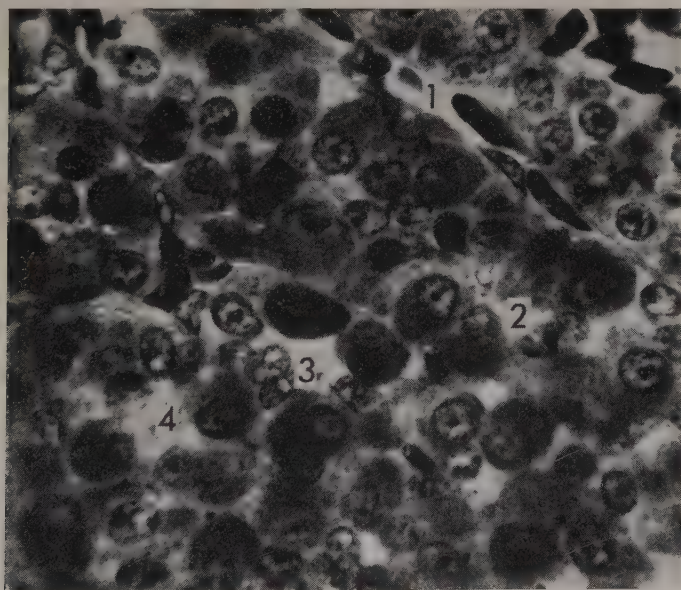


Fig. 20. The same cells as in fig. 19 after the gold had been removed and the section stained in azan. The Ag-cells in the preceding figure are identical with the A_1 -cells, which are very dark after azan staining. Some Ag-negative cells prove to be chromophobes (to the left of the figure 3, for instance). The figures in the photographs mark out corresponding places and thus facilitate the comparison. — Technique see fig. 19. Green filter. 1200 $\times$.

procedure, but in a very special way, for the gold is precipitated in such a state that it looks red or yellow (cf. fig. 17—20). This is different from the Ag-granules of the basophils which are distinctly black after the same treatment. It must be observed that in the A_1 -cells the acidophilic granules themselves are impregnated. In basophils the argyrophilic granules are usually not identical with the chromophilic substance (see below). The A_1 -cells can also be easily identified in celloidin sections stained in the phosphotungstic acid haematoxylin of Mallory, even if the sections measure 40—60 μ (fig. 25). They are restricted to the caudal end of the gland.

In a few specimens the A_1 -cells are difficult to identify in spite of careful fixation. This may be due to degranulation of the cells in connection with the physiological state of the bird, but even in such cases it is often possible to identify them if Bodian impregnation is used. The granules are thus present also in these cases, but they are less stainable. According to Payne (1942) the acidophils are reduced in number in old hens, and such senility changes may explain the absence of distinct A_1 -cells in some of my specimens. In one old black-cock (*Lyrurus tetrix*), for instance, it is not possible to find any distinct A_1 -cells at all in the azan preparations.

In the mag-pies and crows shot in November—December there is another complication. The region usually occupied by the A_1 -cells is here occupied by bluish, violet and red cells, which are all of the same shape and contain granules which look identical in all respects except staining reactions. As mentioned above I have checked the result of the staining in these birds in a series of preparations fixed in different ways and found the same throughout. The series of transitional stages from bluish to red cells is continuous. In the Bodian preparations there is a corresponding series of cells from typical ones with the gold in a red colloidal form to such with more blackish granules. All these cells are, however, in all respects except the colour, typical A_1 -cells and are probably only different functional stages of the same type of cells. At any rate it is impossible to classify the blue cells as basophils, for they have the same coarse granulation as the A_1 -cells, and further the chromophilic granules themselves become impregnated with silver. In the true basophils which are also present in the same glands the argyrophilic granules are not identical with the chromophilic substance. This appearance of the A_1 -cells may be typical of the crows and mag-pies in late autumn, but on the other hand many preparations of passerine birds show a similar curious staining reaction of the A_1 -cells. One young mag-pie from May seems, however, to be essentially similar to those described above.

2. *Basophilic cells.* In azan preparations these cells are, in the majority of cases, easily identified by their blue cytoplasmatic inclusions which have the shape of distinct granules in a few cells but are present as homogeneous masses or diffuse clouds in other cells. The appearance of the basophilic matter in the preparations is no doubt highly influenced by the fixation, a fact pointed out also by previous investigators. The cells are not clearly demonstrated by the resorcin-fuchsin of

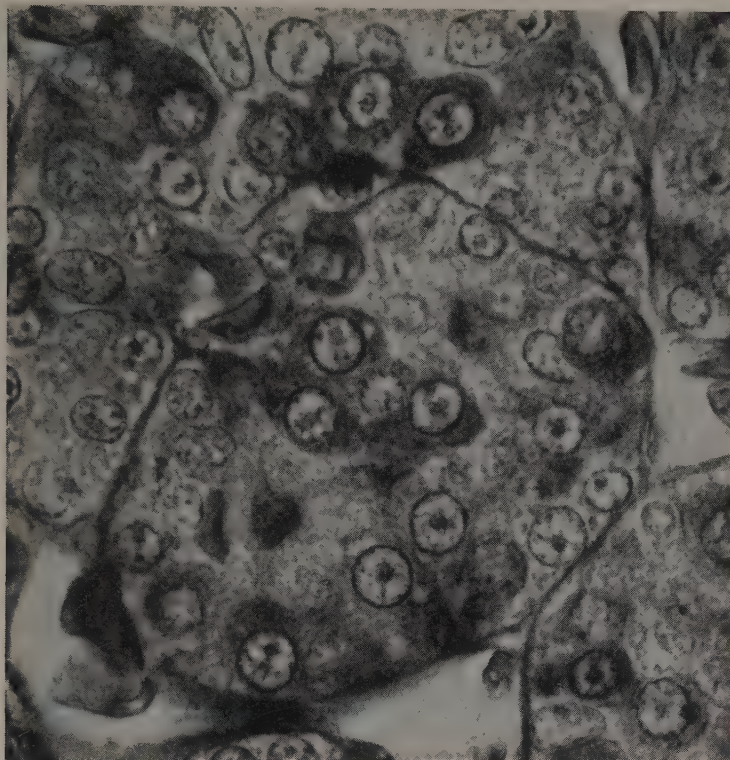


Fig. 21. Strings with basophils (the dark cells) and A₂-cells in the cephalic lobe of a mag-pie (*Pica pica*, ♀ ad., 2. XI.). — Bouin, paraffin 3 μ , azan. Red filter. 1500 $\times$.

Weigert (R § 1561 and 2198), a method which gives beautiful results in some mammals and reptiles. The basophilic cells are often large with rich plasm and a vesicular nucleus which, in many species, looks lighter than that of the acidophils. The basophils show a tendency to concentrate in the rostral end of the gland, the so-called cephalic lobe.

The classification of the basophils causes some difficulties and for several reasons. Sometimes it is very difficult to stain the basophils distinctly, and apart from the cases in which less careful fixation can explain the poor stainability such decrease in the basophilic reaction must be correlated with the physiological state of the bird. Payne (1942) has shown that the basophils gradually change and disappear in old hens, and there are strong reasons to believe that also cyclic changes can explain the appearance of some glands. In addition there are, in all glands, a continuous series of transitional stages from chromophobes to distinctly basophilic cells, and it is difficult to state a definite limit for the cells classified as basophils.

Other complications are of a more principal interest. It was concluded above (p. 44) that the colloid secreted into the colloid acini probably is independent of

the function of true basophils. Now the cells packed with such colloid and prepared to undergo holocrine disintegration may look very similar to the true basophils. In fact, it is impossible to distinguish these types with certainty in thick preparations, and even in very thin sections ($2-3\ \mu$) this distinction is difficult. It is true that the cells producing the acinar colloid contain the homogeneous blue masses in large droplets or vacuoles, whereas the true basophilic cells have the stainable matter more uniformly scattered, often in a granular state. These characteristics are, however, not very easy to use in practice. This, of course, might support the theory that the basophils with a more regular and sometimes granular appearance of the stainable matter are the precursors of the holocrine cells, as supposed by Voigt (1939). I repeat, however, the statement above that the acinar colloid can be produced also in parts of the adenohypophysis where the basophilic cells are absent, particularly in the pars tuberalis. The relatively low frequency of holocrine cells definitely shows that this way of development must be regarded as an exceptional or degenerative process. It is of course possible that true basophils sometimes behave in this way, but the presence of colloid droplets in typical A_2 -cells and chromophobes indicates that other cells also are capable of such development. In fact, the holocrine function can be interpreted as an exceptional accumulation in one cell of the colloid, which is normally secreted in a merocrine way, and the consequent degeneration of the cell.

The Bodian technique reveals some complications which must be considered in this connection. The unquestionable holocrine cells which are packed with colloid and begin to show degenerative changes, never contain any Ag-granules, whereas typical basophils usually do. This, of course, can be taken as an indication of the non-identity of the two cell-types. In most cases the true basophils contain Ag-granules which look completely black in the preparations. The granules never have the confluent appearance or the tendency to aggregate which is characteristic of the basophilic substance. A direct comparison of the cells after Bodian impregnation and azan staining according to the technique described on p. 45 shows that the Ag-granules stain very indistinctly in azan and usually can not be identified with certainty, except in a few cells where they appear as bluish points. Also in sections first impregnated according to Bodian and then stained in azan it can be seen that basophilic substance and Ag-granules are not identical. It is often possible to see blue masses between the black Ag-granules. That Ag-granules and basophilic substance are two different things is also apparent from the statement that some Ag-cells look completely chromophobic in azan preparations, and that apparently normal basophils sometimes lack Ag-granules (fig. 22—23). These cases will be discussed below.

The chromophobes containing Ag-granules are fairly numerous in the series of mag-pies and crows. This may indicate that these "chromophobes" in fact belong to the basophilic line of differentiation, and that this could be shown if an improved technique is used. It is interesting to see that Pearse (1950) when using the periodic acid — Schiff technique for staining the basophils of mammals could classify as

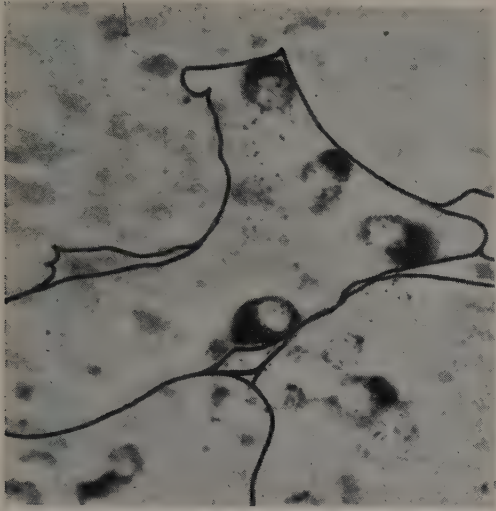


Fig. 22.

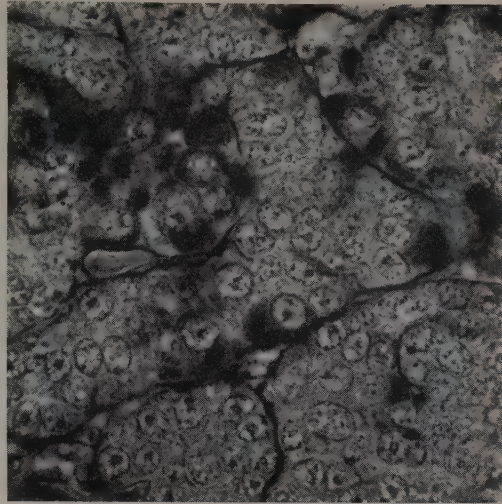


Fig. 23.

Fig. 22. *Pica pica* (ad., 19. X.). Ag-cells and Ag-negative cells in the cephalic lobe of the pars distalis. The membranes around the strings have been drawn with india ink on the photograph to facilitate the comparison with the following figure. — Helly, paraffin 3 μ , Bodian. 1000 $\times$.

Fig. 23. The same area as in fig. 22 after the gold had been removed and the section stained in azan. The Ag-cells in the preceding figure are basophils (black), but there are also some basophils without argyrophilic granules. The light cells in the figure are mainly A₂-cells. — Technique see fig. 22. Red filter. 1000 $\times$.

basophils a large number of cells which in common preparations appear as chromophobes. The technique used by Pearse is considered highly specific and allows an identification of basophils at an early stage of differentiation. Though the chromophobes with Ag-granules found in the pituitary of birds thus may prove to be early basophils, this cannot be said with certainty. It should be pointed out, however, that the prospective basophils acquire Ag-granules long before they actually become basophilic in bird embryos (p. 104).

On the other hand, some cells of the same appearance as typical basophils may lack Ag-granules completely. They are not common, but present in all glands examined with the technique required, that is, in the series of crow and mag-pie pituitaries. I exclude here all the hyperchromatic and vacuolar cells which are prepared for holocrine disintegration and formation of acinar colloid. In some cases two basophils lying side by side and of exactly the same appearance in the azan preparation may differ by the absence or presence of Ag-granules (fig. 22—23).

The relationships between argyrophilic granulations and basophilic matter may seem rather confusing after the above description. Most facts can, however, be explained by the supposition that the Ag-granulation and the basophilic matter are correlated with different functions, and that some cells may have only one of

these functions but that there is a strong tendency to combination of these functions in the same cells. In the histogenetical chapter (IV) it will be shown with a high degree of certainty that Ag-cells are capable of producing the melanophore-expanding hormone even in the absence of basophilic substance. The basophilic substance, or rather, the basophilic cells, seem to be correlated with the thyrotropic and gonadotropic hormones in birds (Payne 1942, 1944, see also chapter VI). It is admitted that this suggestion is too theoretical, but, on the other hand, it is difficult to find another explanation. The histogenetical investigation shows that the Ag-cells can produce the melanophore hormone. The Ag-granules in the embryos, therefore, should not be regarded as some transitional or temporary structure in the basophils, but most probably represent a hormone-producing mechanism of their own. In fact, the Ag-granules seem to constitute a permanent constituent of the cell, since the Ag-cells have a very constant distribution during the ontogeny of the gland (fig. 64) and also in the adult, in which they are most common in the cephalic end of the pars distalis. I exclude from this discussion the granules of the A_1 -cells, which also become impregnated in Bodian, but in a very specific way. These conclusions should, however, be regarded as preliminary and more researches are needed before the complex questions concerning the nature of the basophils can be definitely answered.

3. A_2 -cells and 4. *chromophobes*. These two kinds of cells are treated together here because they are often difficult to distinguish. The A_2 -cells have, in typical cases, distinct though fine granules which stain light red or orange in azan. Their plasm is fairly rich. They are distinctly restricted to the rostral end of the pars distalis. The most typical chromophobes are often situated centrally in the cell strings. They have a very narrow zone of plasm around the nucleus, and do not contain any visible granules or other stainable matter. There are, however, always intermediate types which form a transition between the A_2 -cells and the chromophobes. In some bird pituitaries all the light-stained cells contain granules which stain red in azan, and it may be difficult to find any cells at all which could be clearly classified as chromophobes. This condition has been observed after fixation in various mixtures (Susa, Zenker, Bouin, Helly). In other glands, treated in the same way, all these light cells appear as chromophobes. Since these possibilities are realized in different specimens of the same species, it is evident that we have to do with cyclic changes of some kind. Though it is evident that A_2 -cells and chromophobes may be different stages of the same cells it is certainly correct to keep them apart as far as possible. As mentioned above some "chromophobes" are related to the basophils because of their Ag-granules. Further some slightly granular A_1 -cells form a continuous transition from these cells to chromophobes. It has been generally supposed that the chromophobes are the inactive undifferentiated type of cells from which all the active, granulated cells arise. For this reason it is justified to treat the chromophobes as a cell type of their own.

The chromophobes show a tendency to form aggregates of nuclei, the "Kernhaufen" of German authors, in many specimens. Here the nuclei lie tight together

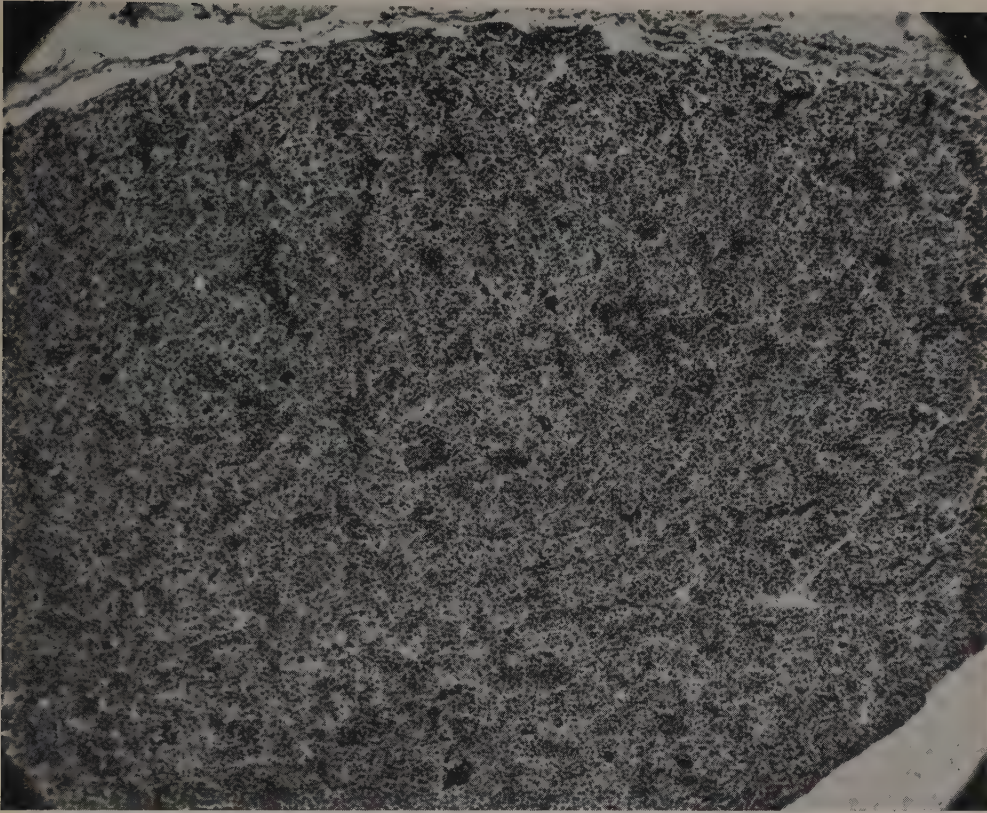


Fig. 24. *Clangula hyemalis* (♂ ad., 28.II.). The cephalic lobe of the pars distalis. The dark accumulations of nuclei are formed by small chromophobes, so called "Kernhaufen". — Bouin, paraffin 6 μ , azan. 110 $\times$.

and the plasma between them is clear and sparse. Formation of such nuclear aggregates are not to be regarded exclusively as a senile degeneration, since they are present in some herring gulls (*Larus argentatus*) even before sexual maturity, and in fowl even earlier (cp. Voigt 1939).

Argyrophilic granules are definitely absent in the A_2 -cells and many chromophobes. As mentioned above some cells looking like chromophobes in azan preparations contain Ag-granules and are therefore probably related to the basophils in some way. In fact, it is difficult to identify the chromophobes in an objective way because of the transitional forms passing to granular cell types.

On the shape of the cells very little has been said. Many authors have observed that the acidophils tend to be more elongated and columnar with the nucleus situated near the end opposite to the basal membrane. The basophils are more isodiametrical, irregular with a more central nucleus. In addition the nuclei of the acidophils are more compact and stainable whereas the nuclei of the basophils are

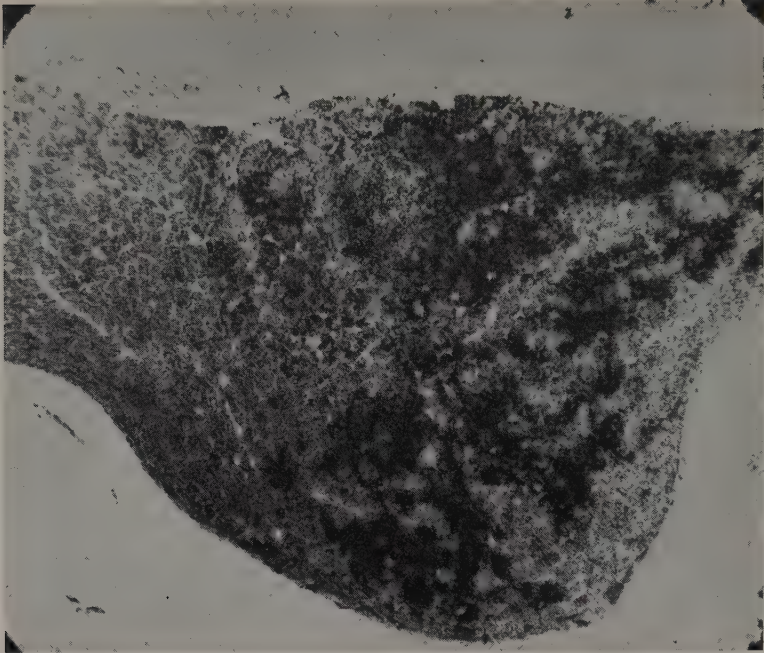


Fig. 25. Median section through the pars distalis of a whimbrel (*Numenius phaeopus hudsonicus*, ♂), the same bird as fig. 6: J. The A₁-cells are here visible as dark clouds in the caudal lobe (right) whereas they are absent in the cephalic lobe (left). — Bouin, celloidin 40 μ , phosphotungstic acid haematoxylin of Mallory. 80 $\times$.

large, vesicular and with few nucleoles. This holds good for fowl and also for most other birds, but the variations in cell shape, nucleoli etc. are so large from one species to another that I have not ventured to describe them. The figures 17—23 illustrate some variations.

Exceptional cell types. In many pituitaries I have found a few cells which do not agree with the classification given above. They are partly hyperchromatic cells of different kinds. Pycnotic nuclei are also often observed.

In a number of glands I also found a peculiar type of cells with large, vesicular nuclei and a light, rich plasm. The cells are very similar to large sympathetic ganglion cells. They lie alone or a few together, always in small numbers. I have seen such cells in the pars distalis of *Strix aluco*, *Clangula hyemalis*, *Regulus regulus* and *Apus apus*. They appear to correspond to the ganglion-like cells described in the armadillo by Oldham (1938), but could not be shown to be of nervous origin.

Pigment granules seem to be rare in the epithelial cells of the pars distalis, I have found considerable amounts of pigment only in *Spheniscus magellanicus* and *Procellaria*. Payne (1942) found pigment frequently in the pituitaries of fowls, especially in very old ones.

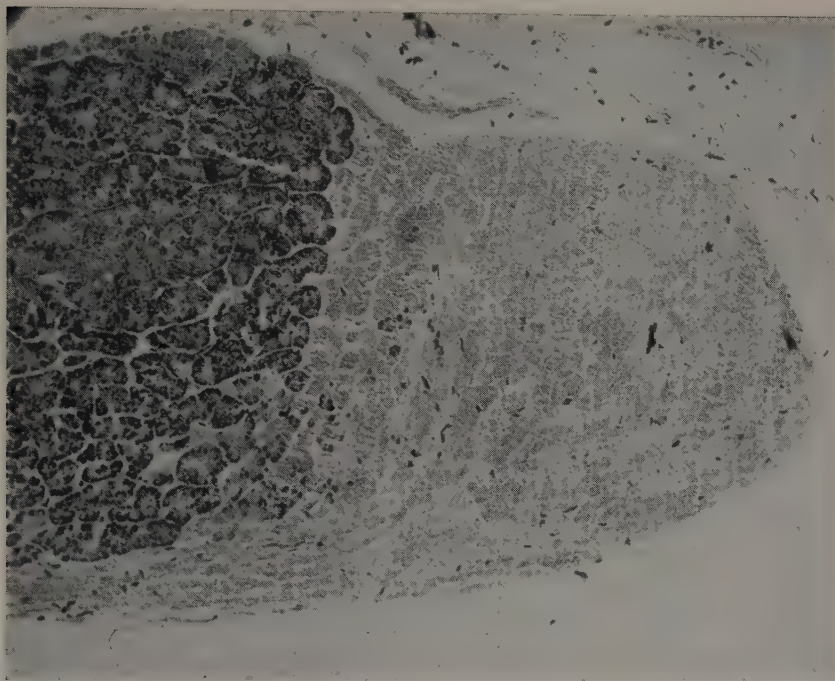


Fig. 26. Paramedian section of the pars distalis of a gull (*Larus ridibundus*, juv., TL 28 cm). The A₁-cells, which are stained black by the iron haematoxylin, are entirely restricted to the caudal lobe (left). — Susa, paraffin 4 μ , iron haematoxylin of Heidenhain. 100 $\times$.

The Histological Subdivisions of the Pars distalis

The distribution of the different types of glandular cells within the pars distalis of birds was treated from a comparative point of view by Rahn and Painter (1940, 1941). They propose the term "avian pituitary pattern" for the typical arrangement of cells in birds. It is characterized by the partition of the gland into two lobes which are histologically different: the caudal lobe, and the cephalic lobe. The most outstanding difference between these "lobes", is that the A₁-cells are restricted to the caudal lobe and the A₂-cells to the cephalic lobe. Usually, there is no corresponding external bipartition of the adult gland. Rahn and Painter (1941) used a material comprising 18 species of birds, and their conclusions therefore claim a more general validity than other statements on bipartition which are based on one or a few species only (Herring 1913, Rahn 1939, Romeis 1940, Painter 1942, Obussier 1948, p. 208).

In my preparations I have been able to confirm the validity of this "avian pituitary pattern" for almost all specimens in which the A₁-cells could be identified with certainty. A few exceptions will be commented on below. For mapping the extension of the two lobes I have mainly used the A₁-cells because they are easily

recognizable after most staining procedures. They could often be identified also in thick celloidin section after staining with the phosphotungstic acid haematoxylin of Mallory (cf. fig. 25, 26). I could thus use practically all section series for this investigation. As is seen in fig. 6, the limit of distribution of the A_1 -cells runs approximately from the point of attachment of the epithelial stalk (if present) to the point of origin of the pars tuberalis. This was also observed by Rahn and Painter, who remarked that this boundary does not correspond to the extension of the embryonic hypophyseal cleft. This is strikingly demonstrated by my embryonic preparations of *Gallus*, *Larus* and *Riparia*, and can also be directly observed in the adult partridge pituitary (fig. 6: M).

Though A_2 -cells and basophils are more or less restricted to the cephalic lobe they are not so useful for distinguishing the limits. They are not so easy to identify as the A_1 -cells and the basophils may occur also in the caudal lobe.

Rahn and Painter report such a bipartition of the gland in 18 species of birds, including Anseriformes, Galliformes, Lariformes, Columbiformes, Apodiformes, Piciformes, and Passeriformes. In my material I have found a distinct histological bipartition in the following genera (cf. also fig. 6).

Struthioniformes: *Struthio*.

Sphenisciformes: *Spheniscus*.

Procellariiformes: *Diomedea*, *Priocella*, *Procellaria*, *Oceanites*, *Pelecanoides*.

Pelecaniformes: *Phalacrocorax*.

Ciconiiformes: *Nycticorax*.

Anseriformes: *Cygnus*, *Anser*, *Branta*, *Anas*, *Clangula*, *Melanitta*, *Somateria*.

Lariformes: *Larus*.

Charadriiformes: *Numenius*, *Calidris*, *Tringa*, *Vanellus*.

Galliformes: *Lyrurus*, *Lagopus*, *Phasianus*, *Perdix*, *Gallus*.

Columbiformes: *Columba*.

Falconiformes: *Coragyps*, *Accipiter*, *Buteo*.

Strigiformes: *Asio*, *Strix*.

Cuculiformes: *Cuculus*.

Piciformes: *Dendrocopos*, *Picus*.

Caprimulgiformes: *Caprimulgus*.

Apodiformes: *Apus*, *Sephanoides*.

Passeriformes: *Leptastenthura*, *Elaenia*, *Xolmis*, *Phylloscopus*, *Sylvia*, *Regulus*, *Turdus*, *Parus*, *Riparia*, *Sitta*, *Emberiza*, *Passer*, *Pica*, *Corvus*.

Apart from a few specimens in which the A_1 -cells could not be observed at all because of insufficient staining methods (*Enicognathus*, *Agapornis*) I found a bipartition in all birds examined except one specimen of *Milvago chimango*. In the gland of the latter, which was sectioned in celloidin, the A_1 -cells are very distinct but they are uniformly distributed throughout the gland. Only a small rostro-dorsal area does not contain any A_1 -cells, but this area is too small to correspond to a cephalic lobe.

In domesticated birds the bipartition sometimes seems to be less distinct, according to literature. Some indisputable A_1 -cells are present in the cephalic lobe of some pigeons (*Columba livia*) in my collection, but they play a minor rôle there, and the bipartition is still distinct (fig. 27). Rost (1940) found a bipartition in



Fig. 27. Paramedian section of the pituitary of a pigeon (*Columba livia*). The bipartition of the pars distalis with the dark A₁-cells restricted to the caudal lobe is distinct. The pars tuberalis is highly reduced in this specimen. The portal vessels are not accompanied by any epithelial strings which could be called a portal zone. — Bouin; injected from the carotis, paraffin 5 μ , azan. 85 $\times$.

this species, but the variations seem to have been fairly great as to the extension of the lobes. And though there is a bipartition of the gland in duck and fowl (Rahn 1939, Painter 1942) the accounts of Payne (1942, p. 80) and Obussier (1948, p. 208) seem to indicate a considerable variation also in these species. This increased variability in hypophysial histology may be due to domestication. Obussier (1948) pointed out that domestication causes a high variability of the pituitary in many animals.

A confusing report on the pituitary of pigeons was given by Schooley and Riddle (1938) and Schooley (1937). They recognized "a posterior-central (juxta-neural) area containing a preponderance of basophilic cells and an anterior-peripheral area containing mostly acidophilic cells". This is just opposite to what I found in my specimens (fig. 27) and also in the pigeons examined by Rost (1939) and by Rahn and Painter (1941). Rahn and Painter paid special attention to this species because of Schooley's and Riddle's results. I think the latter must have

made a mistake owing to the technique used. Their basophilic cells certainly correspond to the A_1 -cells, which thus must have got an unusually high affinity to basic stains.

Koch (1942) and Apor (1942) distinguish three zones in the pars distalis of the pigeon: 1) a mainly chromophobic, superficial zone which is of little interest for the present comparison, 2) a dorso-caudal "Dorsalzone", characterized by basophils, and 3) an oro-ventral "Ventralzone" characterized by acidophils. However, the "basophils" in Koch's paper correspond perfectly to the A_1 -cells of the present account, since they have coarse granules and are stained by common haematoxylin. True basophils in birds have little, if any, affinity to this stain and have indistinct granules. Thus, the "Dorsalzone" and the "Ventralzone" are identical with the caudal and cephalic lobes in the present account. However, I cannot accept Koch's opinion that the A_1 -cells are basophils, for their affinity to azocarmine and iron haematoxylin definitely shows that they are acidophils (cf. Romeis 1940, p. 85 ff.).

V. Soos (1935) writes that he found basophilic cells only in the posterior part of the pars distalis of the turkey (*Meleagris gallopavo*). His fig. 9 seems to indicate, however, that he has mistaken the ends of the gland, and the crosses indicating basophils are present in that part of the organ which must be supposed to be the cephalic lobe. This agrees better with other statements.

As a matter of fact the above list of birds together with that of Rahn and Painter (1941) shows that the term "avian pituitary pattern" is justifiable. This does not mean, however, that the histology of all bird pituitaries is exactly alike. On the contrary, I have found great variations in the appearance and distribution of the glandular cells within the limits given by the bipartition. Since my material does not allow a detailed comparison of many species I shall only give a few examples.

The A_1 -cells are sometimes, as in young specimens of *Gallus*, in adult *Caprimulgus europaeus*, *Coragyps atratus*, in a young *Asio otus* and in an old *Numenius arquata*, very numerous and distinct, and the boundary between the lobes can be drawn from one cell to another. In addition to the A_1 -cells the caudal lobe also contains basophils, which may be very few as in my specimens of *Pica*, *Larus* and *Asio*, but more numerous in most other specimens. In other preparations the A_1 -cells are not predominant in the caudal lobe to such a high degree, but basophils and light-staining cells make up a considerable part of the epithelium. Many light-staining cells in this lobe are similar in structure to A_1 -cells and may be degranulated cells of this kind. They may thus be called chromophobes, but it is of course difficult to state whether they are able to give rise to any type of granular cells, or whether they are determined to form A_1 -cells only. The small chromophobes packed together into aggregates ("Kernhaufen") are more rare in this part of the gland than in the cephalic lobe. Whether typical A_2 -cells are present in this part or not is difficult to decide, because some cells, which look like A_2 -cells in fact may be A_1 -cells in the state of degranulation or early differentiation. They are, anyhow, not common.

The cephalic lobe usually looks more chromophobic in Azan preparations. The cells are, in most glands, chiefly A_2 -cells or chromophobes. One of these kinds may

predominate completely or they can be seen together in approximately equal numbers. Basophils are always fairly numerous and are often more or less restricted to this part. A_1 -cells occur sometimes, especially in the region next to the caudal lobe, but are always rare (e. g. in some specimens of *Columba*, *Somateria*, *Anser*). The small chromophobes are very common in some pituitaries and form large aggregates. Since some of these differences may depend on cyclic and age variations which cannot be investigated on the basis of my material I shall not enter into a detailed description. Some information can be found in the papers by Payne (1942), Schooley and Riddle (1938), Koch (1942), and Apor (1942).

The Pars Tuberalis

Morphology

The pars tuberalis of birds consists of 1) a thin layer of cells lying within the pia mater on the surface of the brain, the *pars tuberalis proper*, and 2) a *portal zone* with strings of epithelial cells which connect the pars tuberalis proper with the pars distalis. The portal zone is continuous with 3) the *pars tuberalis interna*, which is paired and has fused intimately with the pars distalis (fig. 28). Histologically the pars tuberalis interna forms a transition between the pars tuberalis and the pars distalis, and it is discussed here because of its embryonic development from the lateral lobes.

The pars tuberalis proper in most cases is more or less paired. It forms one membrane of epithelial tissue on each side of the eminentia reaching from the chiasma and tractus opticus rostrally to the posterior side of the infundibular stem caudally. The two parts have usually fused in the median line at two points: 1) in the middle of the eminentia whence the portal zone is emitted, and 2) behind the infundibular stem. In this way a collar is formed around the stem (fig. 28). In many cases the median parts of the eminentia are not covered by the pars tuberalis rostrally and caudally of the portal zone, but there are regularly some points of fusion just behind the chiasma. The pars tuberalis of each side reaches far beyond the limits of the eminentia mediana upwards along the sides of the diencephalon (fig. 1, 28).

In most birds the portal zone is independent of the infundibular stem and runs directly to the pars distalis, in contrast to the conditions in most mammals in which it runs along the stem. In one specimen each of *Elaenia albiceps* and *Pyrrhula pyrrhula*, however, I found a portal zone closely attached to the stem in the same way as in mammals (fig. 29). The portal zone is usually unpaired and median in adult birds but in the remarkable pituitary of the humming-bird *Sephanoides* the portal zones of each side are completely separated. Here the pars distalis is closely attached to the eminentia mediana and even follows the furrows on its surface. The pars distalis thus completely separates the portal zones in this species (fig. 6: S).

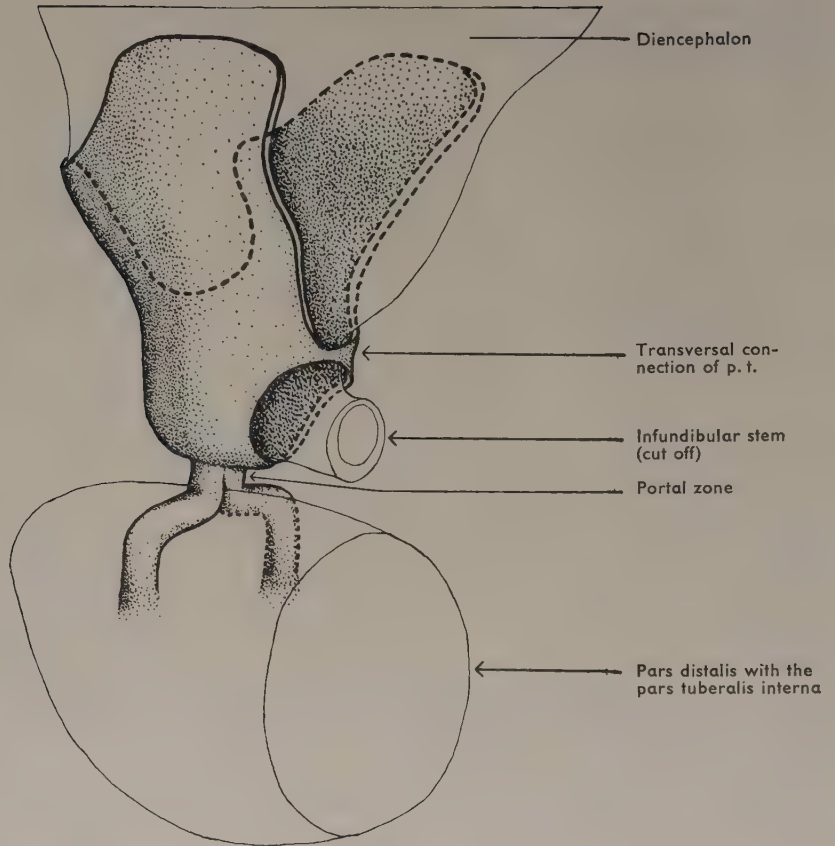


Fig. 28. Diagram showing the extension and subdivisions of the pars tuberalis in birds. The pars tuberalis is stippled, the brain and the pars distalis are indicated by delicate lines. The gland is seen from the left and behind. — Graphic reconstruction from a series of horizontal sections through the sphenoid region of *Parus atricapillus*. The plane of reconstruction forms an angle of 45° with the median plane. The pars tuberalis interna was not distinct in this specimen and is therefore only roughly indicated. 73 $\times$.

The pars tuberalis interna (fig. 30, 31) is a transitional zone between the pars distalis and the pars tuberalis. When the portal zone reaches the surface of the pars distalis the strings are divided into two bundles which run transversally to each side and then turn more ventrally, filling the furrow between the cephalic and caudal lobes. These parts are called the pars tuberalis interna because they are parts of the tuberalis which have been incorporated in the pars distalis. From an embryological point of view the pars tuberalis interna in birds corresponds to the basal parts of the lobi laterales (chapter IV).

The pars tuberalis interna is very distinct in my specimens of *Larus*, *Lyryrus*, *Cygnus*, *Melanitta*, *Coragyps*, *Phalacrocorax*, *Spheniscus* and *Procellaria*, but can

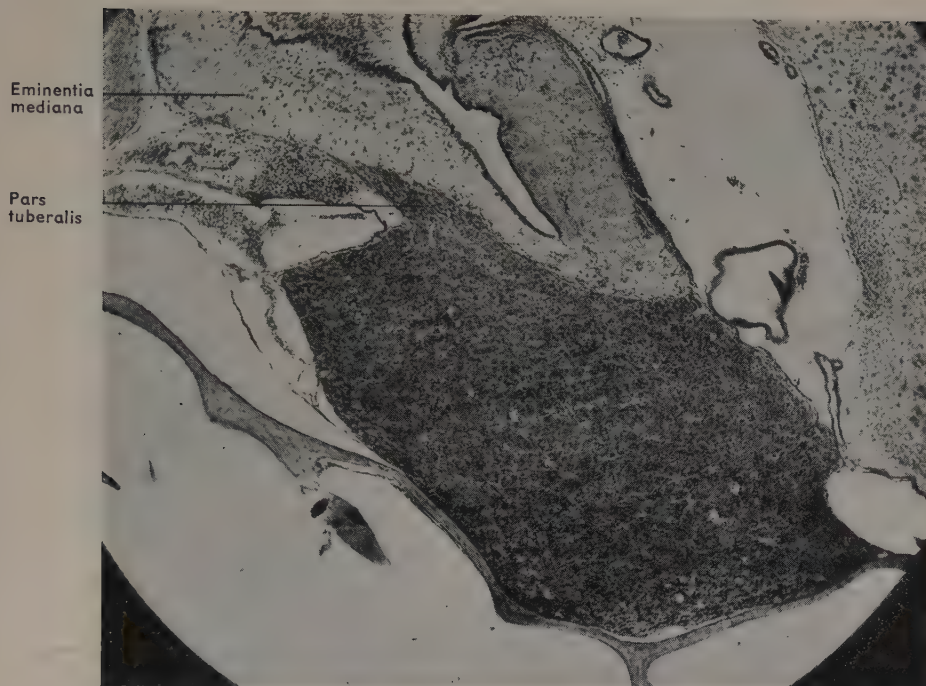


Fig. 29. Atypical pars tuberalis in *Elaenia albiceps*. Note that the portal zone is closely attached to the infundibular stem, and compare fig. 30. Median section. — Bouin, paraffin 8 μ , haematoxylin of Ehrlich and eosin. 85 $\times$.

be distinguished in most birds as a more chromophobic, transversal string on each side coming from the portal zone.

The pars tuberalis is present in all the birds which I have examined though it is very unequally developed. Thus, in all the Procellariiformes it forms several layers of cells and cell strings on the surface of the brain, and the portal zone is here a compact glandular body of considerable dimensions (fig. 32). The other extreme is the humming-birds (*Sephanoides*), in which the portal zone consists of a few cells only on each side. This zone is paired and the fusion of the anlagen has been checked by the pars distalis which is in direct contact with the eminentia. The pars tuberalis proper is extremely difficult to distinguish in the humming-birds, but there is one single and fairly continuous layer of cells with the same extension as in other birds. The cells are, however, so flattened that they look like endothelial cells and their plasma is difficult to distinguish in azan preparations, because they may be mistaken for reticulocytes belonging to the pia. Their argyrophilic granulations in Bodian preparations as well as their power to produce colloid droplets reveal their true nature.

Most other birds occupy an intermediate position between the two extremes referred to above. The brain is covered by an epithelium consisting of 1—4 layers



Fig. 30. Median section, showing the connection between the portal zone (p.z.) and the pars tuberalis interna (p.t.int.) in a gull (*Larus argentatus*, ♀ juv.). — Bouin, injected from the carotids, paraffin 8 μ , azan. 95 $\times$.

of typical cubic cells (fig. 33, 34). The portal zone varies considerably, and in most birds consists of fairly few (5—15) epithelial strings which cannot be said to form a compact organ because a considerable part of the zone is occupied by the portal vessels and connective tissue. In *Phalacrocorax* and *Coragyps* the portal zone is nearly as compact as in the Procellariiformes, but, on the other hand, the portal zone in some specimens of *Columba livia* and in most Anseriformes is very poorly developed, containing only a few or no epithelial strings at all (fig. 27).

It should be pointed out here that the variations in the pars tuberalis are considerable even in different specimens of the same species. Thus, for instance, the tuberalis areas of each side of the diencephalon may not fuse behind the infundibular stem — this was the case in specimens of *Struthio*, *Pica*, *Anas*, *Numenius*, *Accipiter*, *Columba*, *Dendrocopos*, *Passer* and *Calidris*. In other specimens of some of these species such a fusion has taken place, so the difference is certainly not very important (*Anas*, *Columba*, *Pica*).

In this connection it is necessary to comment some statements in Nemec's paper of 1950. Nemec's conclusion that the pars tuberalis proper may reach rostrally to the chiasma and even cover a small zone of its ventral surface is certainly

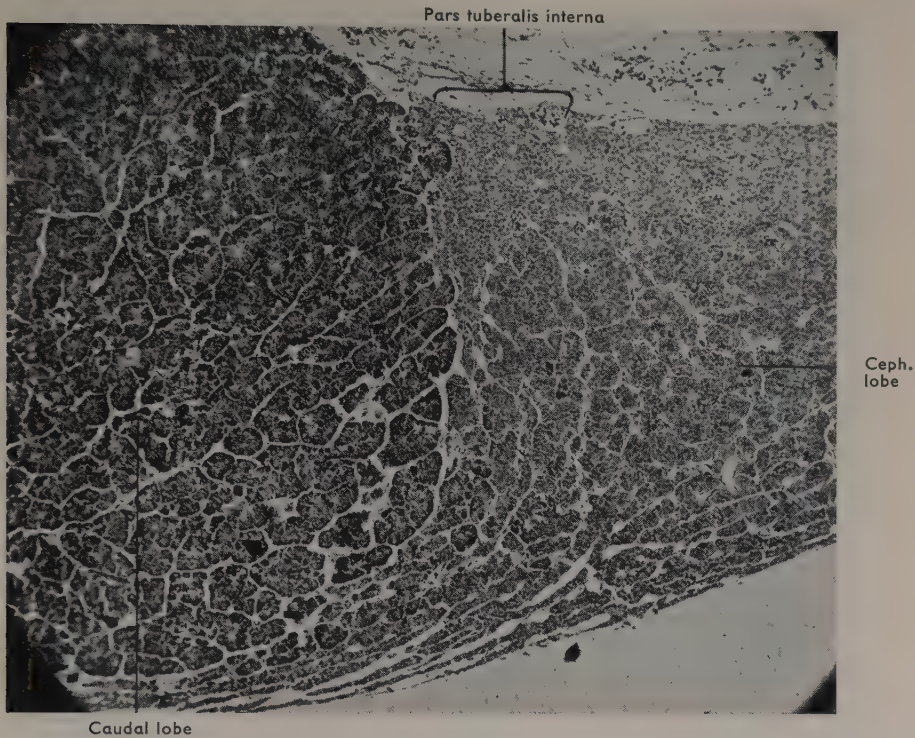


Fig. 31. Paramedian section through the pars distalis of a young, nearly full-grown gull (*Larus ridibundus*) showing the situation of the pars tuberalis interna between the caudal and cephalic lobes. The pars tuberalis interna is visible as a triangular area of light cells with distinct nuclei. — Susa, paraffin 4 μ , azan. 90 $\times$.

correct. The tuberalis may fill the furrow between the chiasma and the diencephalon and thus cover a narrow zone of the chiasma, and this seems to be a general rule, but variations are great even within the same species, e. g. in *Columba livia*, where in some specimens the pars tuberalis is almost completely reduced.

Nemec is further of the opinion that a fusion of the tuberalis sheaths behind the infundibular stem takes place only in occasional cases — he could find such a fusion only in two of his seven bird species. He also quotes a number of authors which have not described such a ring-shaped tuberalis. In my material there is some variation in this respect as mentioned above, but in the majority of cases a complete fusion could be stated behind the stem. I also refer to Rahn and Painter (1941) who found that the pars tuberalis envelops the stem in their 18 bird species.

Obviously Nemec has overlooked the portal zone in some of his specimens and drawn it distinctly only in the two species of *Palaeornis* (Abb. 8: f and g). Though this zone sometimes may degenerate so that only few strings of epithelium are present among the portal vessels it is important from a comparative point of view since it indicates the embryological relationships. In addition this zone, especially

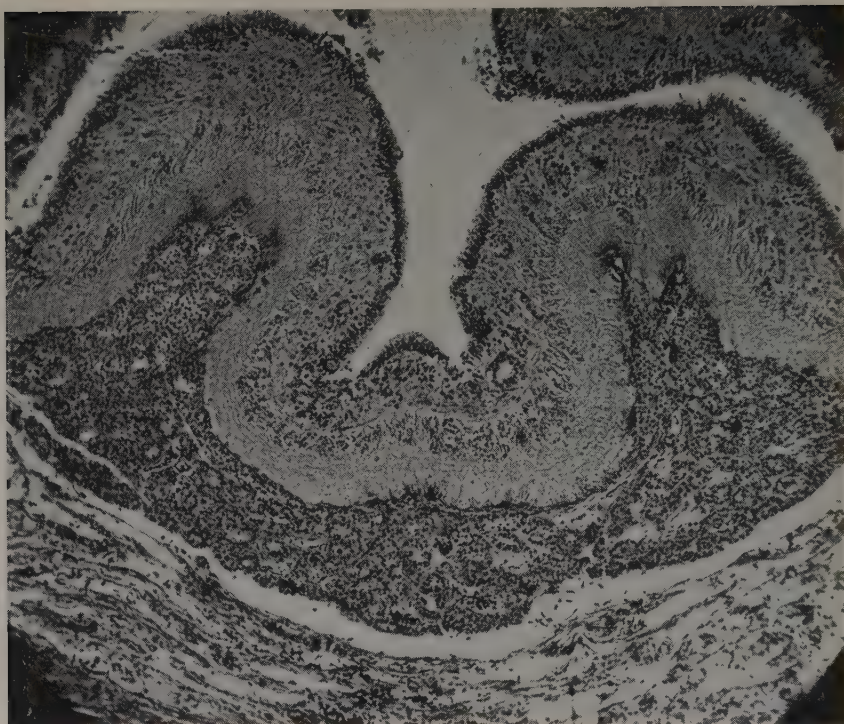


Fig. 32. Strong pars tuberalis in *Diomedea melanophrys* (♀ ad.). Transversal section showing the eminentia mediana and the pars tuberalis proper. — Bouin, paraffin 8 μ , azan. 90 $\times$.

its vessels, is probably the most indispensable part of the tuberalis from a functional point of view. This portal zone was drawn perfectly already by Müller (1871) in a pigeon, by Krause (1922), by de Beer (1926) and by several recent investigators, e. g. Rahn and Painter (1941).

Finally, the fact that median sections contain less tuberalis tissue than paramedian ones is emphasized by Nemec. This condition is explained by the embryonic development. As the pars tuberalis develops from paired lateral lobes there is originally no tissue present in the median plane and a fusion of the two parts does not take place until late embryonic or post-embryonic stages.

Histology

The cells in the pars tuberalis, as in the pars distalis, are arranged in anastomosing strings and lamellae. If we first consider the pars tuberalis proper, the more or less continuous sheath of cells on the brain surface, this is divided into very flattened strings or lamellae by delicate membranes, but there are usually no membranes separating the cells in planes parallel to the brain surface. This only occurs if the pars tuberalis consists of 3—4 cell layers or more. The flattened

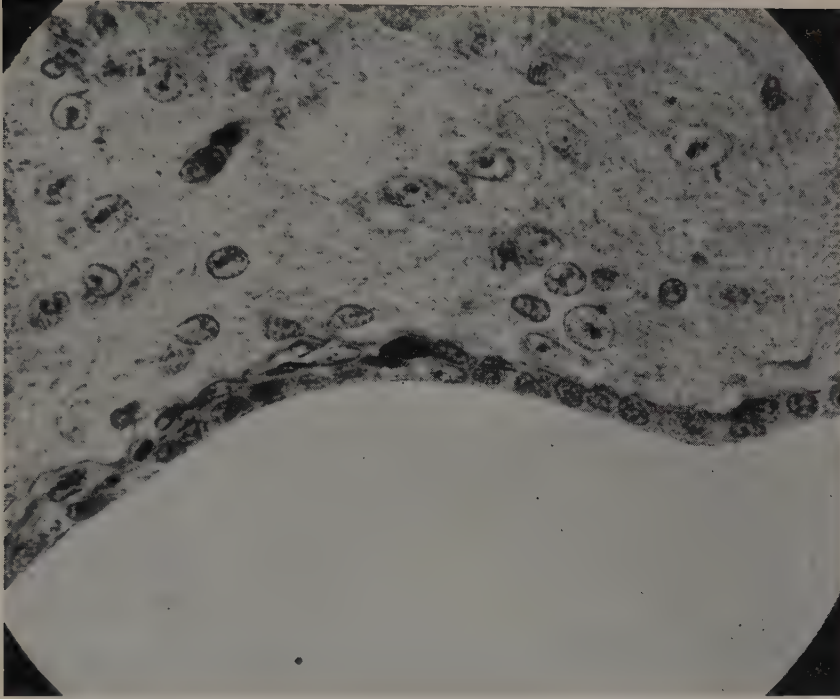


Fig. 33. Single-layered pars tuberalis on the surface of the diencephalon of a swift (*Apus apus*, fledgling). — Bouin, paraffin 8 μ , azan. 830 $\times$.



Fig. 34. Photograph showing the thin, single-layered tuberalis of a goose (*Anser anser*, ♀ ad.) in relation to the eminentia mediana and adjacent parts of the diencephalon. The tuberalis is intimately attached to the surface of the former but is separated from the latter by loose connective tissue. The median plane is indicated by the double arrow. Transversal section. — Bouin, paraffin 8 μ , azan. 85 $\times$.

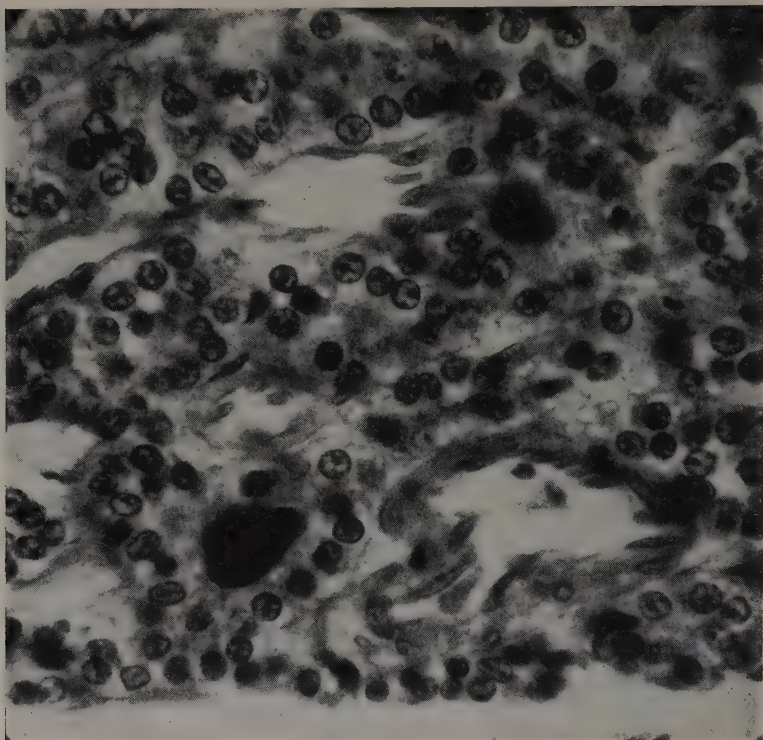


Fig. 35. *Diomedea melanophris* (♀ ad.). Two colloid acini in the pars tuberalis proper. — Bouin, paraffin 8 μ , haematoxylin of Ehrlich + eosin. Green filter, 750 $\times$.

strings which constitute this part of the gland are surrounded by delicate reticular membranes of the same argyrophilic type as those in the pars distalis. Brainwards these reticular membranes are in immediate contact with the surface of the eminentia (the intima piae) or, in places, separated from it by capillaries belonging to the primary plexus of the hypophysiportal system. Outside the eminentia, higher up along the sides of the brain, there is some space between the internal basal membrane of the tuberalis and the intima piae. This space is then occupied by fibres, among which there are also several coarse, collagenous ones, and further by some vessels. These fibres may sometimes form a fairly dense connective tissue membrane which separates the epithelium from the brain surface here. Along the periphery of the pars tuberalis this membrane is continuous with the pia mater, but so is also the external reticular membrane of the pars tuberalis. The external reticular membrane of the pars tuberalis is here and there connected with the dura and the vessels by the scattered fibres which traverse the subdural space (see fig. 34).

The arrangement of the cells in the portal zone is somewhat different. Here we find long strings of cells which are all orientated along the portal vessels, that is, from the brain to the pars distalis. The strings are surrounded by argyrophilic reticular membranes also here, but in addition there is much collagenous tissue,

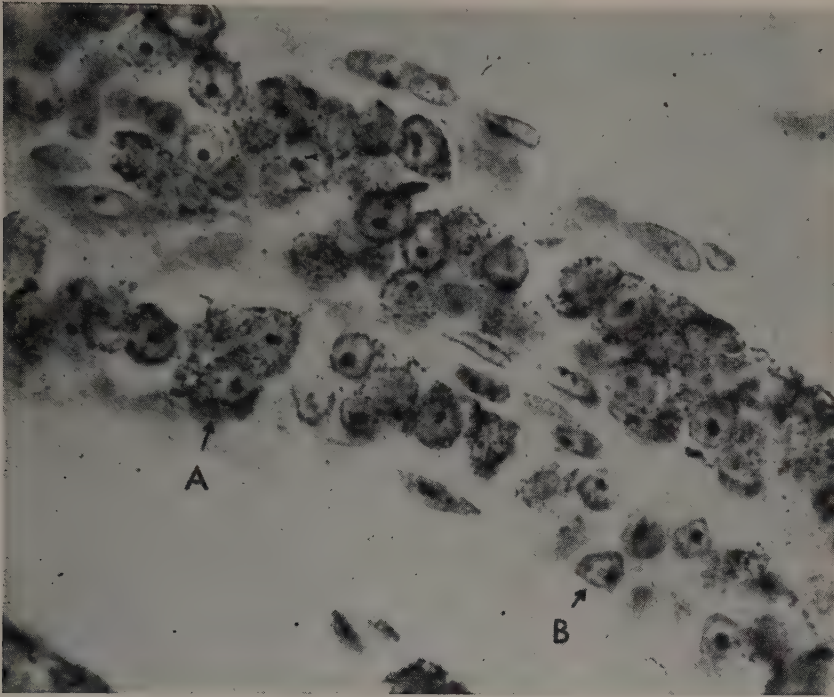


Fig. 36. Ag-cells in the portal zone of the pars tuberalis of a young *Larus argentatus*, demonstrated by silver impregnation. At A there are some granular cells and B shows some non-granular ones. — Bouin, injected from the carotids, paraffin 8 μ , Bodian. 1300 $\times$.

which fills the spaces between the portal vessels and the cell strings. The pars tuberalis interna looks very like the pars distalis as regards the arrangement of the cells.

In the *Procellariiformes* the pars tuberalis has no character of a vestigial organ. The pars tuberalis proper is here remarkably thick and in its central parts consists of several strata of epithelial strings, supplied with a rich capillary net (fig. 35). The portal zone is very mighty and compact and the epithelium is here the prevailing component. In fact, the arrangement of the cell strings and the general appearance of the tuberalis in these birds does not differ very much from that of the pars distalis, may be the collagenous fibres and the vessels are not so abundant in the latter. The *Procellariiformes* are unique also in showing an invasion of epithelial cells into the eminentia mediana. This was distinctly observed in *Diomedea melanophris*, *D. epomophora*, *Pelecanoides magellanicus*, *Priocella antarctica*, *Procellaria aequinoctialis* and *Oceanites gracilis*. The membranes between the surface of the eminence and the nearest tuberalis strings have disappeared in two symmetrically situated areas, one on each side of the eminentia, and the tuberalis cells have spread into the nervous tissue. Some of these cells are to be found far from the surface and may lie quite isolated. It should be pointed out, however, that in the

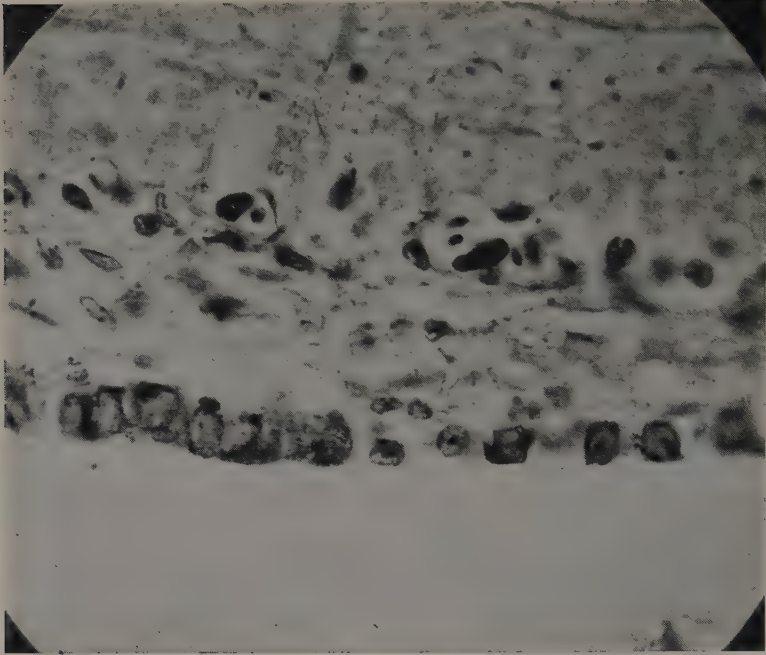


Fig. 37. Ag-cells in the single-layered pars tuberalis on the surface of the eminentia mediana of a *Coragyps atratus*. — Bouin, paraffin 8μ , Bodian. $800\times$.

small pituitary of *Oceanites* this invasion was only indicated and had not advanced so far.

This immediate contact between the tuberalis cells and the eminentia mediana is interesting since a similar invasion takes place in some lizards, a fact which I have seen in numerous preparations, and mentioned also by Gaupp (1893) and Baumgartner (1916), but not verified since then. In *Lacerta agilis*, for instance, the epithelial anlagen of tuberalis lose contact with the pars distalis and arrive at the surface of the eminentia, where they sink down into the nervous tissue and form a small island of cells on each side, inside the pial cover. The situation of the two intracerebral cell masses in *Lacerta* on each side of the eminentia seems to correspond to the two areas of invasion in the Procellariiformes.

Colloid acini are common also in the pars tuberalis, but here as in the pars distalis the amount of colloid varies extremely, also within the same species. The colloid sphaerules are surrounded by the epithelial cells and show the same staining reactions as in the acini of the pars distalis (fig. 35). The colloid acini in the pars tuberalis are usually common in such glands which also contain abundant colloid in the pars distalis. For this reason and also because of the identity in structure I think the acini in the pars distalis and the tuberalis have the same physiological significance in birds. The origin of the acini in the pars tuberalis has not been observed in detail, but I have often found minute blue droplets smaller than a

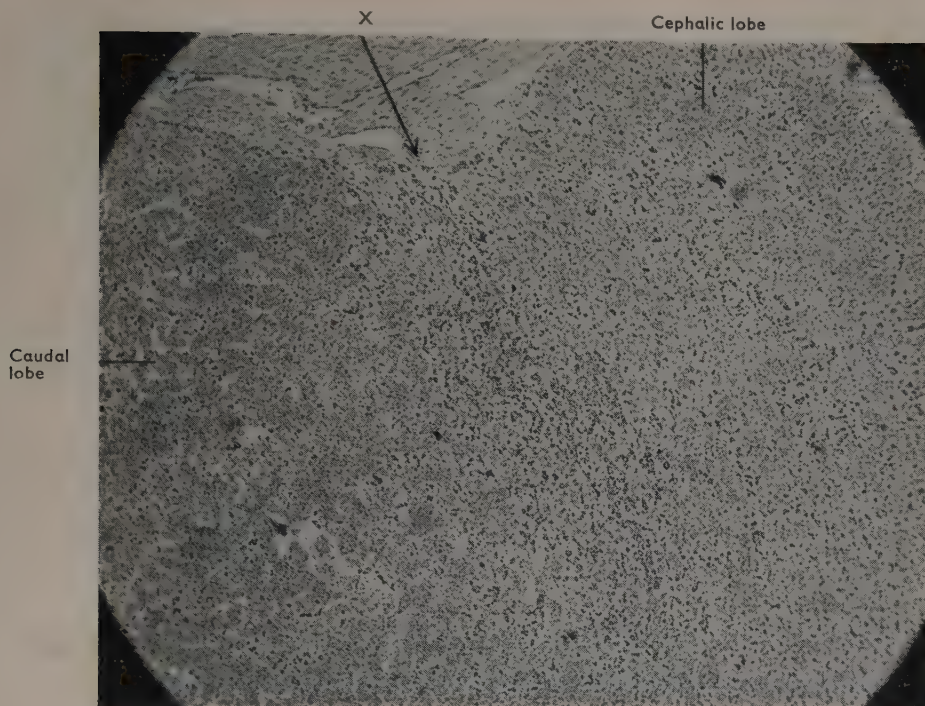


Fig. 38. Paramedian section through the pars distalis of a young swan (*Cygnus olor*, TL 70 cm.). The course of the pars tuberalis interna is indicated by the arrow X, and is characterized by the density of the Ag-cells. It is not distinct in an azan preparation of this specimen. — Bouin, paraffin 4 μ , Bodian. 90 $\times$.

nucleus between the epithelial cells. These small colloid droplets may be the first stage in the development of the acini. Since no distinct chromophilic cells are present, the colloid production could not be confined to basophils or acidophils in this part of the gland.

The Procellariiformes which have a mighty and apparently functional tuberalis vary in the same way as regards the amount of colloid. One of the *Diomedea* specimens contains numerous large follicles so the organ almost looks like a thyroid, another specimen completely lacks the colloid. Curiously enough, also one of the small *Sephanoides* specimens shows distinct traces of colloid formation in its vestigial tuberalis. The blue droplets here lie between the extremely flattened cells of the pars tuberalis proper. Since the colloid droplets are several times as large as the cells themselves they bulge out into the surrounding tissues.

The glandular cells of the pars tuberalis proper and the portal zone are essentially the same and may therefore be described together. The plasm of the tuberalis cells of birds is very indistinctly stained in azan preparations. They appear almost hyaline or, in most cases, filled with an indistinct granular substance which may take a reddish, violet or bluish colour depending on the degree of differentiation.

There is, however, a slight difference in the stainability from one cell to the other — some cells become darker and contain more distinct granules. After Bodian impregnation these latter cells can be shown to contain agyrophilic granules, which fill the cell more or less completely (fig. 36, 37). Though these cells are rarely so packed with black granules as the basophils of the pars distalis, they show, however, that we have a cellular differentiation also in the pars tuberalis. These Ag-granulated cells have been found in all bird pituitaries impregnated according to Bodian — in the extremely vestigial tuberalis of the *Sephanoides* specimens as well as in the well developed tuberalis of *Diomedea*. They are, however, rare in the tuberalis of *Columba livia* and *Anser anser*. I think this granulation, just as the colloid acini, indicates a secretory activity of the pars tuberalis. On the other hand, the great variations of the tuberalis in birds and its absence in some reptilia show that the organ is not indispensable.

The cells of the pars tuberalis interna are partly, in some birds exclusively, typical tuberalis cells. In other specimens there are also many pars distalis-cells, i. e. basophils, and A₂-cells. This part is, therefore, a transitional zone from the pars tuberalis to the cephalic lobe of the pars distalis from a histological point of view. In a specimen of *Caprimulgus europaeus* also the portal zone contains several distinct basophils in its upper part. The Ag-cells are exceptionally common in the pars tuberalis interna (fig. 38).

Whereas other authors have only seen one type of cells in the pars tuberalis of birds, Rahn (1939) reported that both basophils and acidophils appear in the tuberalis of the chick embryo between 14 and 18 days of incubation but that they later disappear. The acidophils are said, however, to be less granular than those of the pars distalis. As a third type of tuberalis cells Rahn distinguishes the chromophobes. Though I have been able to confirm Rahn's embryological statement I cannot distinguish more than two types of cells in the pars tuberalis of the adult bird: the Ag-granulated and the Ag-negative.

Structures Resembling a Pars intermedia

In my bird pituitaries I have never seen any structure which could be interpreted with certainty as a pars intermedia corresponding to that of mammals, reptiles and amphibians. In the majority of cases the pars distalis of birds is separated from the neural lobe by a thick layer of connective tissue, and the distance between the lobes may sometimes be considerable. In a few rare cases the separating membrane is very thin (fig. 39), but with some exceptions which will be discussed below, the epithelial and nervous structures do not meet immediately (cf. also Nemec 1950 a). However, in some pituitaries structures occur which suggest an intermedia, and a few such cases will be discussed here.

In many birds the coarsely granulated acidophils (A₁-cells) of the caudal lobe do not extend to the periphery of the gland along the juxta-neural surface, and there

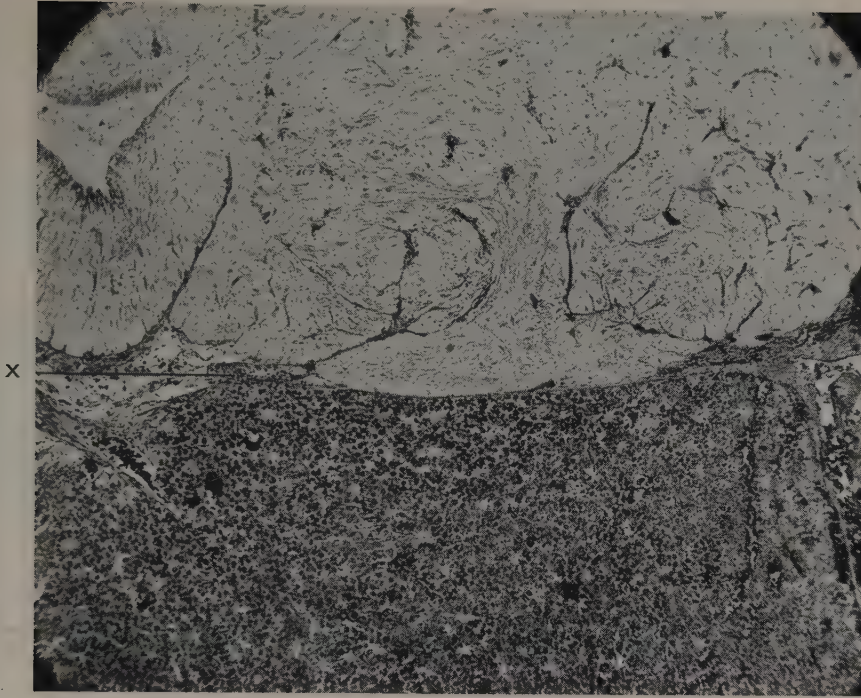


Fig. 39. Median section of a gull pituitary (*Larus fuscus*, ♀, about 2.5 years old), showing the intimate contact between the neural lobe and the pars distalis in this specimen. At X an epithelial string is even directly connected with the nervous tissue, but this is not clearly visible in the photograph. — Susa, paraffin 6 μ , azan. 100 $\times$.

is a zone consisting of more or less chromophobic cells, sometimes also basophils. As there are no specific intermedia cells in this zone there is, however, no reason to call it intermedia. Further, some strings of more or less chromophobic cells may sometimes project from the pars distalis in the neighbourhood of the neural lobe, but such strings may be found in the pituitary capsule also far from the neural lobe and certainly have nothing to do with an intermedia.

One kind of such strings is of special interest. In one specimen of *Larus fuscus*, one of *L. argentatus* and one of *Columba livia* a string of epithelium penetrates the membranes and ends directly in the neural lobe tissue. Very few cells at the end of this process are chromophobic, the other cells look like those in the adjacent part of the caudal lobe. In the specimen of *Coragyps atratus* there are three such strings which are in immediate contact with the neural lobe, but the strings consist of A_1 -cells, chromophobes and basophils as the normal epithelium of the caudal lobe. This is important, for a close contact with the neural lobe is necessary for the differentiation of the intermedia in amphibians (Blount 1945, Hegre 1946). In the said birds there is an immediate contact but still no intermedia.

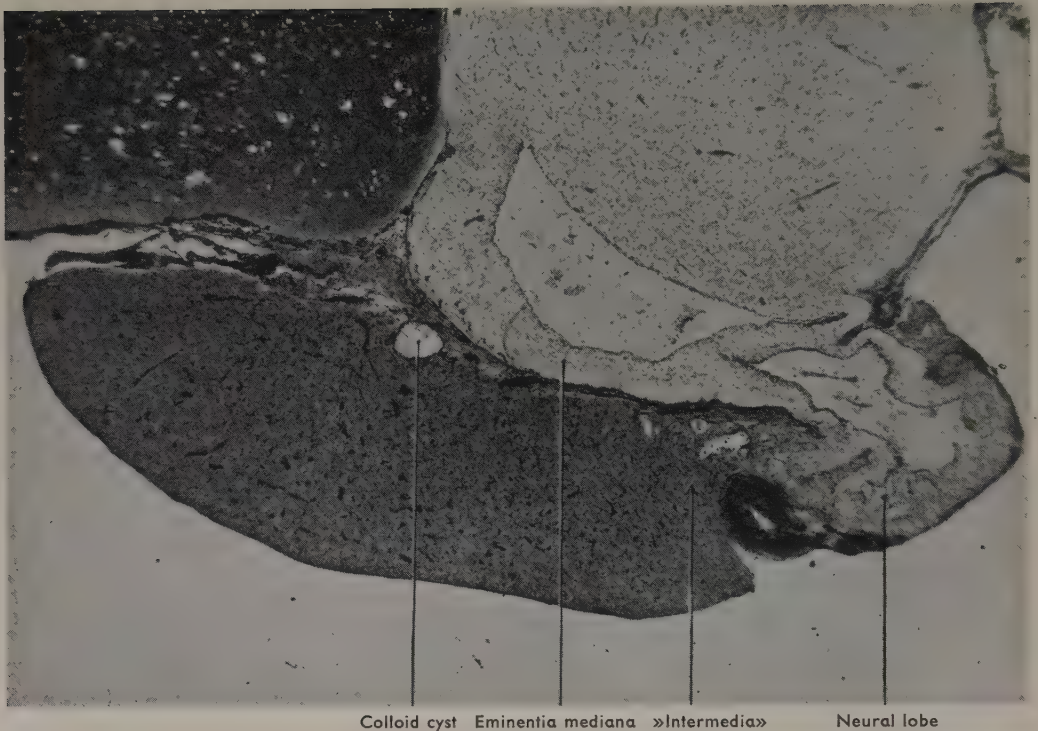


Fig. 40. Median section of the pituitary of a black-cock (*Lyrurus tetrrix*, ♂ ad.). Note the intermedia-like zone near the neural lobe. — Bouin, paraffin 8 μ , azan. 29 $\times$.

The three specimens of *Lyrurus tetrrix* which allow detailed examination have a modified area along the juxta-neural surface (fig. 40). As stated above some birds have a chromophobic zone here, but in the black-cocks this area is characterized by numerous large cysts — just as in the zona intermedia of man. As the contact with the neural lobe is fairly intimate (the separating membrane is thin in some places) this strongly suggests an intermedia with large Rathke's cysts. The cells surrounding the cysts are, however, typical caudal lobe cells and the cysts themselves have developed from colloid acini and thus have nothing to do with Rathke's cysts. The origin of the cysts is apparent from the numerous transitional stages from common small acini near the periphery of the area to large cysts in its centre.

The intermedia-like structure in the black-cocks is probably derived from the same wall of Rathke's pouch as is the true intermedia of other animals. It may thus be homologous with an intermedia from a strict morphological point of view. The intermedia must, however, be defined on a histological and functional basis, otherwise the term is of little value (cf. discussion on p. 153). As the zone in *Lyrurus* lacks specific intermedia cells I consider the term intermedia inadequate in this case.

The absence of a structural and functional intermedia in birds is strikingly demonstrated by the physiological experiments of DeLawder, Tarr and Geiling (1934) and by Kleinholz and Rahn (1939, 1940). They found the highest concentration of melanophore hormone (intermedin) in the *cephalic* lobe of the chicken pars distalis, not in the caudal part which morphologically corresponds to the intermedia of other vertebrates.

The absence of the intermedia in birds has been confirmed by most recent investigators, but contradictory statements have repeatedly occurred in the literature (e. g. Pokorny 1926, Haller v. Hallerstein 1934, v. Soos 1935, Fésüs 1936, Schooley 1937, Tilney 1938, Schooley and Riddle 1938, Voigt 1939, Rost 1939). In many cases it is apparent that the authors have seen the pars tuberalis but used the term intermedia for it. Rost stated that the "intermedia" developed from the lateral lobes and therefore corresponds to the pars tuberalis — a fact pointed out by himself in the text. Also Schooley (1937) and Schooley and Riddle (1938) observed the pars tuberalis in the pigeons and considered it "the nearest approach to an intermediate lobe to be found in birds". Voigt (1939) used the corresponding term (Zwischenlappen) for the pars tuberalis in fowl, but observed that it arises from the lateral lobes.

Haller v. Hallerstein (1934, pp. 293—299) has described the embryonic and adult adenohypophysis in birds as if an intermedia were present. His figure of an adult duck pituitary (fig. 276, *Anas boschas*) has been copied from Gentes. It shows the pars tuberalis, and, in addition, a caudal area along the neural lobe which is called intermedia. Either the specimen drawn must have been extremely abnormal or the figure must be wrong, for no intermedia is present in the duck pituitaries I have examined, nor did Painter (1942) find any such deviations from the general avian pattern in the same species. The part called intermedia in Haller v. Hallerstein's description of fowl embryos (his figures 269—271) is the aboral lobe of Rathke's pouch and does not correspond to the part called "intermedia" in the adult. As will be shown in the next chapter the aboral lobe gives rise to the caudal lobe of the adult — that is, *the part which does not produce any considerable amounts of intermedin*.

I repeat my introductory statement that I have never seen any structure in birds for which the term intermedia is justified.

CHAPTER IV

The Embryology of the Adenohypophysis

The material on which the investigation of the pituitary development is based is listed in table 2 (p. 21). I have confined myself to three species, viz. *Larus ridibundus*, *Gallus gallus*, and *Riparia riparia*. The development of *Gallus* is fairly well known, at least from a morphological point of view, and I therefore only summarize my observations on the morphogenesis of this species, while the two others are treated in more detail. Though morphogenesis and cellular differentiation of the gland are intimately connected I found it necessary to separate them into two subchapters, the former giving the outlines necessary for the latter.

Morphogenesis

Literature

Rathke (1838, 1839) stated that the hypophysis of mammals, birds and reptiles has a double origin: one part is formed by the brain rudiment, and the other part from the pouch of the oral epithelium named after him. The criticism of Reichert (1840, 1861) and others made Rathke alter his opinion and in his "Entwicklungsgeschichte der Wirbeltiere" (1861) Rathke admits that the pouch may not contribute to the gland, but probably disappears. Avian material, preferably embryos of *Gallus*, were used by these authors and also by other debaters in the discussion (e.g. Baer 1828, 1837, Dursy 1868, 1869). The statements from this time have, however, mainly historical interest.

Müller (1871), in a study covering all vertebrate classes, was able to confirm Rathke's original opinion. He used duck, fowl and starling as representatives of the class Aves. Now the double origin of the gland was more generally recognized, but as late as 1885 the existence of Rathke's pouch was denied by Albrecht.

In the next period after Müller's work some of the fundamental problems were recognized, and they were later discussed intensively even during the first decades of the 20th century. Mihálikovics (1874, 1875, 1877) stated that the pouch of Rathke is formed on the rostral side of the buccal membrane and that it must therefore be ectodermal in the fowl. The entodermal pocket behind this membrane was described by Seessel (1877) and was believed to contribute to the fowl pituitary

by some authors (cf. Atwell 1915), but Atwell found that the bud from Seessel's pouch included in the pars distalis is small in *Gallus*. Saint-Remy (1895), Economo (1899) and Bruni (1914) did not find any anatomically distinct contribution of this kind to the pars distalis of the same species. In *Anas* the entodermal pouch was said to contribute with as large a part as the ectodermal pouch (Pfeiffer 1925). This species has been examined embryologically also by Lups (1929) who was not able to decide the question, and by De Beer (1925 and 1926), Friedman (1934) and Painter (1938, 1942). Friedman definitely ascribes to the gland a purely ectodermal origin, and recent investigators of other species seem to hold the same view. Rost (1939) studied the domestic pigeon carefully and was able to state that the pituitary in this species is purely ectodermal, i. e. it is formed by Rathke's pouch exclusively. Also modern investigations on fowl (Atwell and Sitler 1918, Pfeiffer 1925, Lillie 1930, Rahn 1939) have arrived at the same conclusion for this species.

One important step towards a detailed examination of the morphogenesis of the adenohypophysis was the discovery of the "lobi laterali" of Rathke's pouch in the chick embryo (Rossi 1896). They were observed also by Economo (1899, "Seiten-sprossen") and their further development into the pars tuberalis was followed by Tilney (1914), and by Atwell and Sitler (1918). Their statements have later been confirmed by many authors. De Beer (1925 and 1926) collected some evidence for a fusion of the lobi laterales with medial processes from the praemandibular cavities in some animals. The only bird in which this was said to occur was the duck. As the lobi laterales thus should serve as the outlets of the praemandibular somits he compares them to the proboscispores in lower animals. This view was then worked out further by Gislén (1930). The rôle, if any, of the praecordal mesoderm in the formation of the pars tuberalis in the duck was critically reinvestigated by Friedman (1934), who was not able to confirm De Beer's theories. In fowl the praemandibular cavities disappear early and no "proboscis pores" are formed (De Beer 1926), and Rost (1939) did not observe any such pores in the pigeon.

A nomenclature for the different parts of the hypophysial anlage was introduced by Woerdeman (1914). He distinguished a caudal anlage which he called Rathke's pouch, and an anterior complex of primordia, including the lobi laterales, the "Vorraum" and the "Mittelraum". Later it has been shown that the lobi laterales etc. in birds are formed secondarily from the original pouch and thus are not separate from it originally. Lups (1929) introduced this terminology for the pituitary of the duck and the fowl.

Since De Beer's monograph (1926) considerable work has been done, and the development of the pituitary is now fairly well known for three species of birds, viz. *Gallus gallus domesticus* (Atwell and Sitler 1918, Pfeiffer 1925, De Beer 1926, Lillie 1930, Voigt 1939, Atwell 1939, Rahn 1939), *Anas platyrhynchos domestica* (De Beer 1926, Lups 1929, Friedman 1934, Painter 1942) and *Columba livia domestica* (Rost 1939). Some notes on other species have also been published, e. g. on

Sturnus vulgaris (Müller 1871), *Fringilla* (Sasse 1886), *Anser* (Mihálikowics 1875, Müller 1871), *Nycticorax* (Watanabe 1934), *Uroloucha* (Watanabe 1935), *Passer* (Bruni 1914), *Serinus* (Stellwaag 1912).

Nomenclatural Remarks

There has been some confusion in the literature concerning the terminology for the different parts of Rathke's pouch, and it is therefore necessary to state what is meant by the terms used in the present paper. Woerdeman's (1914) terminology was supposed to have a general validity within all vertebrates, but it is written in German and can hardly be translated directly. Further, Woerdeman often used the same terms for the lumina and the surrounding walls (e.g. Rathkesche Tasche, Vorraum, Mittelraum) and this has caused some confusion. Since many terms have been used in more than one sense it proved necessary to introduce some new ones.

First a few remarks on the terms used to describe the orientation of the hypophyseal anlage and the surrounding structures:

Oral and *aboral* denote direction towards the oral epithelium and from it respectively. Sometimes, especially if dealing with the structures near the brain, I have used *ventral* and *dorsal* as synonymes, referring to the orientation of the neural tube in the diencephalic region.

Rostral and *caudal* refer to directions towards the chiasma and towards the medulla oblongata respectively. As synonyms I have used *anterior* and *posterior*. Caudal thus does not refer to the longitudinal axes of the posterior body but to the axis of the neural tube which is bent in the head region.

The following terms have been used for the adenohypophyseal anlage of amniotes:

Rathke's pouch refers to the entire ectodermal invagination which gives rise to the adenohypophysis (Seessel's pouch not included). Woerdeman (1914) and other authors used this term for the top region of the anlage only, the aboral lobe in the present paper (cf. fig. 41).

In a more advanced stage the basal portion of the pouch is constricted and forms the *epithelial stalk* (Atwell 1939), and a lateral constriction divides the rest of the pouch into two lobes: the *oral lobe* and the *aboral lobe*. Parker (1917) proposed the terms "proximal" and "distal", but the term "distal lobe" could easily be confused with the term "pars distalis" and should therefore be avoided. Neither does it seem appropriate to use the term "Rathkesche Tasche" or Rathke's pouch for the aboral lobe, since most modern authors use this term in a wider sense for the whole anlage.

The lumina corresponding to the oral and aboral lobes are called *oral lumen* and *aboral lumen* resp.

The oral lobe often presents a median bud directed rostrally, and this bud is called *anterior process* here (Vorderer Fortsatz, Woerdeman). The corresponding lumen is called *anterior diverticulum* (Vorraum, Woerdeman). The paired buds from the oral lobe are called *lobi laterales*.

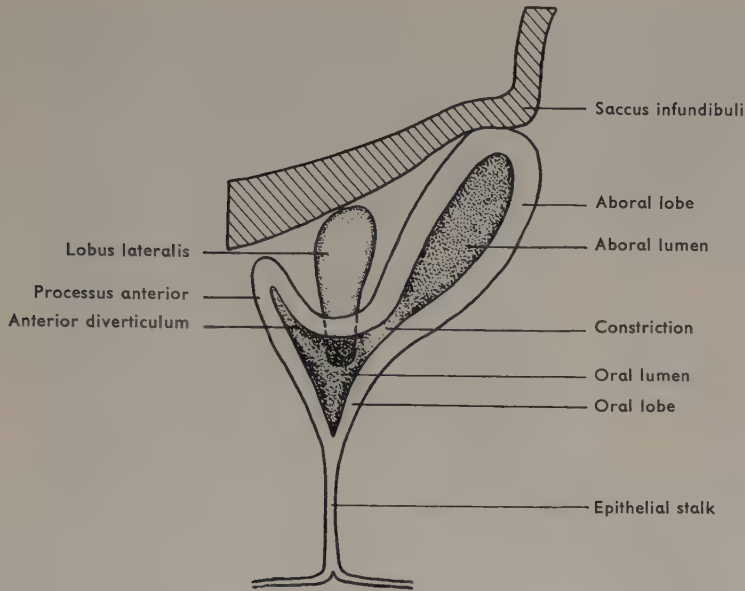


Fig. 41. Generalized diagram of the Rathke's pouch in amniote vertebrates showing the terminology used in the present paper.

Larus ridibundus

I have described the development in embryos of this species first, because here the embryonic structures are often preserved for a very long time. It is possible therefore to find out the relationship between different parts of Rathke's pouch and the structures of the adult gland with a high degree of certainty. The very young embryos are not numerous, but this does not play any considerable rôle in the discussions since the most important transformations take place in somewhat older embryos.

In one specimen with 6—7 pairs of somites there is still no distinct Rathke's pouch. The medullary tube is not closed.

Embryo 7.5 mm. The praemandibular cavities are distinct and large, connected by a transversal string of dense mesenchyma. The top of the notochord is connected with this string by a distinct row of mesenchymatic cells (fig. 42). Rathke's pouch is well developed and presents distinct though small lateral lobes on each side near the base. In addition there is an unpaired diverticulum from the caudal surface. Rathke's pouch is distinctly separated from Seessel's pouch which is almost as large. The identification of the latter is definite since small remnants of the oral membrane are still left in a few places.

Embryos 9.4—14 mm. The praemandibular cavities have disappeared, and the tip of the notochord approximates Rathke's pouch but without complete fusion.

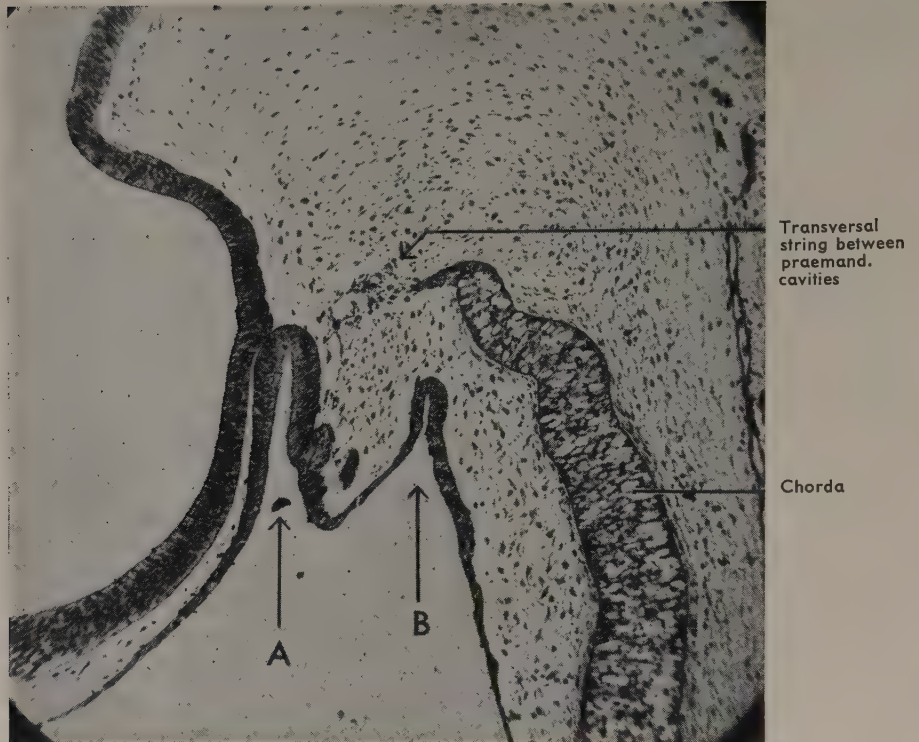


Fig. 42. *Larus ridibundus*, embryo CR 7.5 mm. The median section shows Rathke's pouch (A), Seessel's pouch (B), and the relation to the transversal connection between the praemandibular cavities. — Zenker, paraffin 6 μ , azan. 115 $\times$.

Although the point of the notochord in some cases sticks in the epithelium of the pouch it is always separated from the epithelial cells by distinct connective tissue.

The Rathke's pouch has grown out and become constricted at the base so the first indication of an epithelial stalk is visible. The top of the pouch which is attached to the brain surface is bent caudally from the point where it meets the brain. The walls of the pouch have begun to proliferate except the part of the wall which is attached to the infundibular process. This wall which in other animals gives rise to the intermedia remains single-layered until fairly late stages. The lateral lobes have grown out and their tops are close to the brain but have not yet got in immediate contact with it.

The lumen is still continuous in the median parts and in the lateral lobes. It shows two lateral dilatations, corresponding to similar dilatations of the external contour (cf. fig. 43 and 44):

- 1) an *aboral lumen* in the top region of the pocket, corresponding to the aboral lobe.
- 2) an *oral lumen* in the region of the lateral lobes, corresponding to the oral lobe.

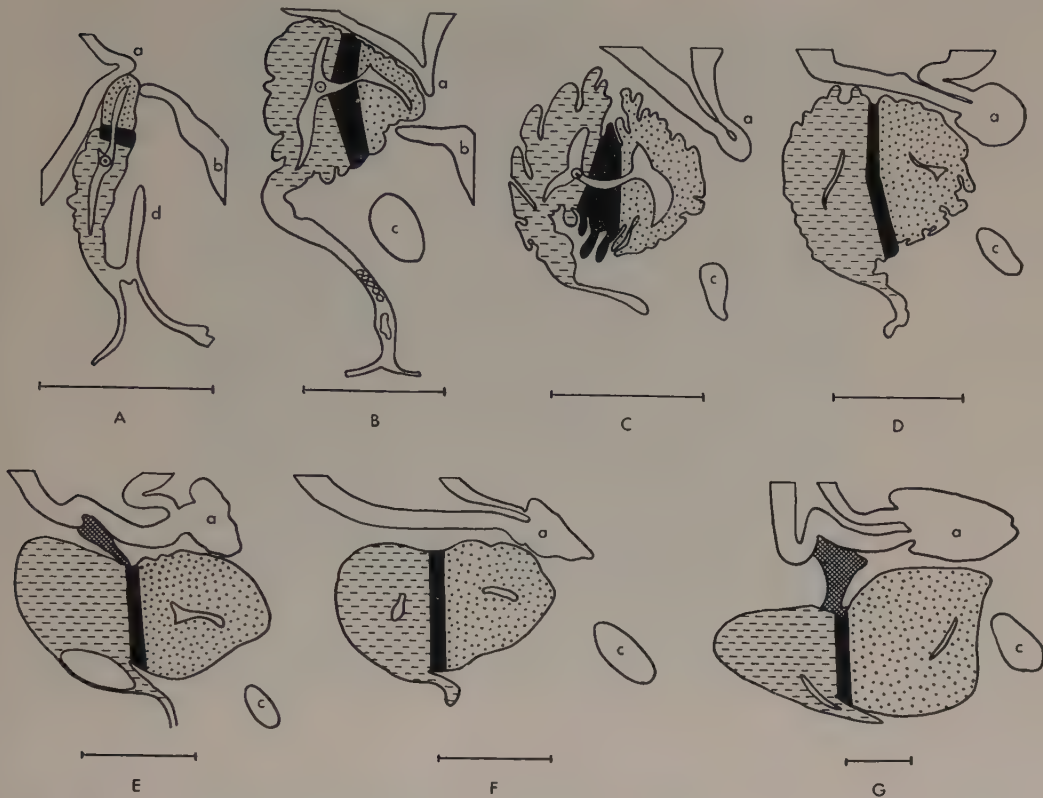


Fig. 43. *Larus ridibundus*. Camera lucida drawings of median sections showing the morphogenesis of the adenohypophysis. Stippled = aboral lobe, short lines = oral lobe, black = tissue derived from the constricted part between the lobes, crossed lines = pars tuberalis. a = saccus infundibuli, b = notochord, c = intercarotic anastomosis, d = Seessel's pouch.

A = CR 14 mm, B = CR 20 mm, C = CR 28, TL 45 mm, D = TL 60 mm, E = TL 78 mm, F = TL 89 mm, G = young, TL 28 cm.

It should be noted that the embryos D—G are exceptionally favourable for a morphological interpretation because of the preservation of the lumina. In many embryos the lumina are less distinct or even absent at these stages. The black area could be perfectly delimited only in A—C thanks to the presence of a lumen in the constricted part. In D—G its breadth is arbitrarily chosen; its main course is, however, distinctly marked by its relation to the lumina and to the lateral lobes, which fill the furrow on each side between the oral and aboral lobes. The bladder in the oral lobe of E is cystically developed and probably does not correspond to a true oral lumen.

The magnification is shown by the line under each figure, representing 0.5 mm.

The constriction between these dilatations is not distinct in a median section, since it narrows the lumen mainly from the sides. Just under the proximal dilatation there is a second constriction of the pouch causing the development of the epithelial stalk.

The fixed points which can be recognized in the anlage now and which are useful for interpreting the structures in older embryos are:

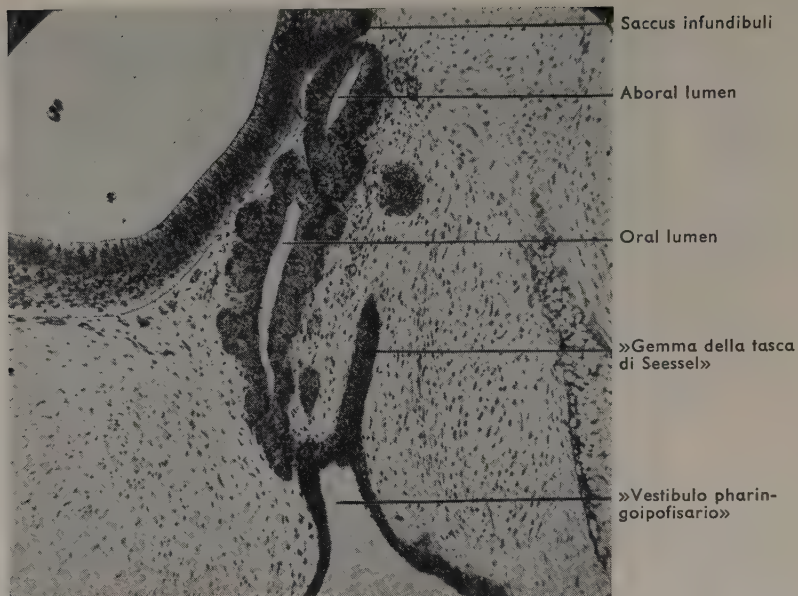


Fig. 44. *Larus ridibundus*, embryo CR 14 mm. Paramedian section. Note the compact bud from Seessel's pouch (gemma della tasca di Seessel of Bruni) and the lateral extension of the oral and aboral lumina. The Seessel's pouch is not perfectly median here. — Zenker, paraffin 6 μ , azan. 95 $\times$.

- 1) The oral and aboral lumina and the constriction between them.
- 2) The points of origin of the lateral lobes. They arise from the oral lobe, from the part nearest the constriction.
- 3) The zone of contact with the infundibular process ("intermedia").
- 4) The point of attachment of the epithelial stalk to the anlage.

The Seessel's pouch has a wide lumen in the 9.4 mm embryo, and is connected with Rathke's pouch by a median membrane of epithelium. In the older embryos Seessel's pouch is shallow and seems to regress (fig. 44). In all embryos, however, it has given rise to a long, solid bud, undoubtedly corresponding to the "gemma della tasca di Seessel" found in *Gallus* by Bruni (1914) and Atwell and Sitler (1918), in *Columba* by Rost (1939) and in *Anas* by Lups (1929). The Seessel's pouch and Rathke's pouch do not open independently into the oro-pharynx but into a common pocket, which in agreement with Bruni may be called "Vestibulo pharingo-goipofisario". In all embryos, however, the Seessel's pouch is situated fairly near the pharyngeal epithelium, the Vestibulo does not grow out, and the epithelial stalk is thus formed mainly by the ectodermal pouch. I have no reason to assume that the entoderm (Seessel's pouch) contributes to the formation of the hypophysis.

In one of the embryos (12.3 mm) there is a bridge of epithelium from the Seessel's pouch to the Rathke's pouch. Such bridges or other modes of fusion between the two pockets may be fairly common in birds, for they have been observed by several

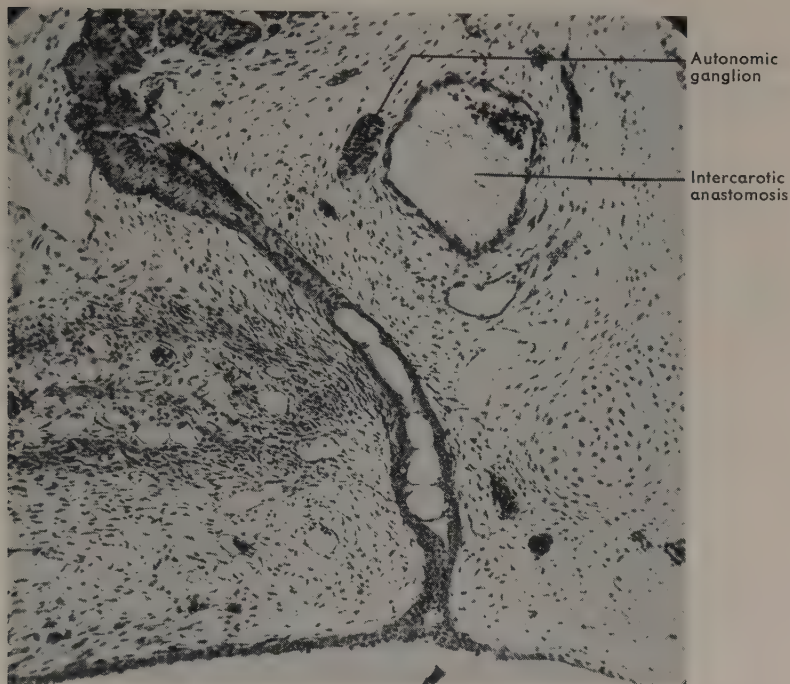


Fig. 45. *Larus ridibundus*, embryo CR 20 mm. Median section of the epithelial stalk showing the vacuoles which occur when it degenerates, and its S-shape. Note the ganglion near the carotis. — Susa, paraffin 6 μ , azan, 110 $\times$.

authors (Saint-Remy 1895, Economo 1899, Bruni 1914, Atwell and Sitler 1918) and I have similar observations from *Gallus* (p. 93). Saint-Remy (1895) even thought that Seessel's pouch opened into the Rathke's pouch through an epithelial tube similar to the compact bridge mentioned above. This tube would correspond to the nasopharyngeal duct in *Myxine*. However, these structures are extremely variable and are certainly of doubtful anatomical value.

Embryos 17—20 mm (fig. 43 B). In these embryos the pouch has been considerably transformed. It is bent at an approximately right angle, the very point of the angle is near the origin of the pars tuberalis. This bending of the distal parts of the pocket seems to be directly caused by the dislocation of the brain which is apparent during this part of the development. Whereas in younger embryos the surface of the brain in contact with the aboral part of the pocket is roughly perpendicular to the oral epithelium, it is later turned about 90° or more. This means that it later runs roughly parallel to the oral epithelium and the top of Rathke's pouch points caudally or even ventro-caudally. There are, however, certainly also other movements which influence the development of the pouch. The base of the stalk in the 17 mm embryo is dislocated caudally in relation to the brain, and in the 20 mm embryo it has assumed the S-shape typical of older stages. These dis-

locations of the epithelial stalk may be due to a movement of the diencephalon and the glandular anlage rostrally in relation to the pharyngeal epithelium, but also to the pressure from the rostral side caused by the development of the cranial basis (see fig. 45). It is, however, always difficult to decide whether only mechanical forces are involved in a transformation like this, or if also specific growth potencies in the epithelium play a rôle.

The Seessel's pouch and its compact process, distinct in earlier stages, is now practically absent. As the younger embryos show, there is a common "vestibulo pharingo-ipofisario" in which both Rathke's and Seessel's pouch open, the latter, however, with a very reduced lumen. This vestibulo seems to form the very basal part of the epithelial stalk, but, as shown in earlier embryos, the major part of the stalk must be a "pedunculo vestibulo-ipofisario" according to Bruni's terminology, that is, it is formed by Rathke's pouch only. Seessel's pouch is, however, not identifiable in the embryos between 17 and 20 mm, and the question how much of the stalk is formed by the vestibule therefore could not be answered. In one of the embryos there is a small knob on the lower part of the stalk. Maybe it is a remnant of the entodermal contribution. Some authors investigating birds have spoken of a disintegration of the compact bud from Seessel's pouch (cf. Lups 1929, Atwell 1915) and even published figures of cell groups in the neighbourhood of the carotic anastomosis which are said to be the remnants of the Seessel's pouch. I have seen such groups of cells in my preparations too, but they are clearly sympathetic ganglia, typically differentiated already at this early stage (fig. 45). It is true that the bud of Seessel's pouch (*gemma della tasca di Seessel*) is directed towards the carotic anastomosis or towards a point just behind it, but I have never seen it disintegrate and it would be sensational if these ganglia are formed by Seessel's pocket, that is, by the intestinal epithelium. I am convinced, however, that the "Epithelzellgruppen" near the carotis drawn by Lups (1929, Abb. 8) are identical with the ganglia I have seen. Rost has found an "accessory hypophysis" in this region in a pigeon which may be a derivate of Seessel's pouch, but he regards it as an accidental structure and I agree with him.

The epithelial stalk is still continuous in these embryos but has partly lost its lumen. The lateral lobes contain a lumen in their terminal parts which are in close contact with the brain, and begin to proliferate. The basal parts of the lateral lobes are compact, and their origin in the oral lobe can only be marked out approximately if the direction of the cell strings is followed.

The most interesting transformations have taken place in the glandular anlage proper. The top of the pocket still attaches to the infundibular process so this point is still identifiable. This wall, the "intermedia anlage" is still single-layered, whereas the other walls have begun to proliferate. The lumina of the glandular body are still preserved and make it possible to identify all parts of Rathke's pouch with certainty. The aboral lumen is very broad from side to side and has developed a median diverticulum from its originally rostral (juxta-neural) side (fig. 46, 47). The constriction between the two lobes is very narrow and the lumen is

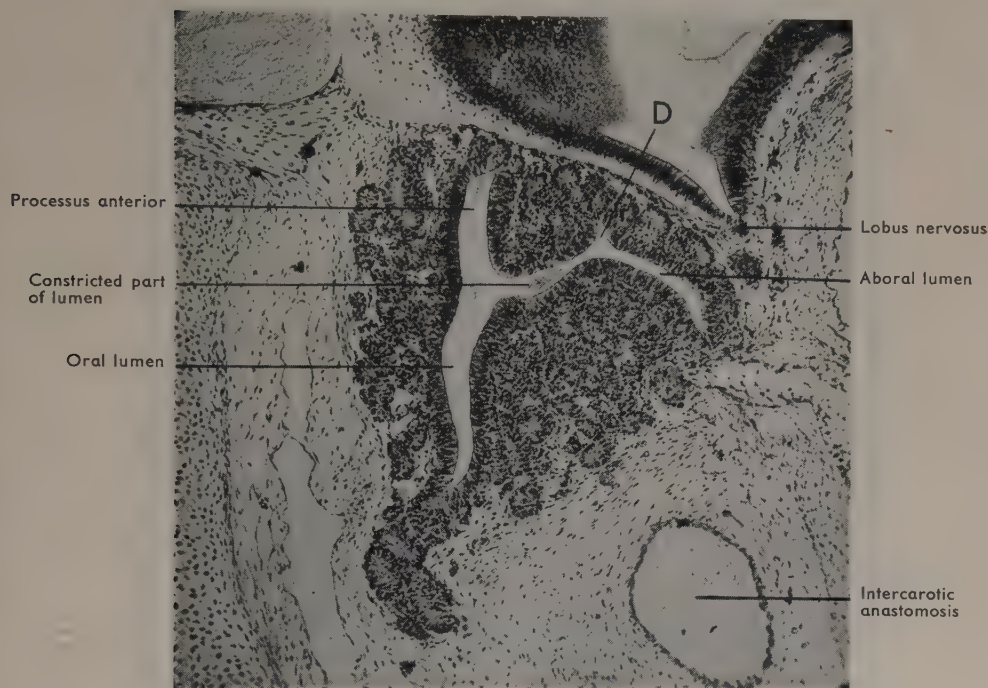


Fig. 46. *Larus ridibundus*, embryo CR 20 mm. Median section showing development of lumina. D = rostral diverticulum from the aboral lumen. — Susa, paraffin 6μ , azan. $84\times$.

here only about $20\text{--}30\mu$ broad. The oral lumen is also wide from side to side and has a very large and constant anterior diverticulum which is situated between the bases of the lateral lobes and undoubtedly is identical with the "Vorraum" mentioned by Woerdeman (1915) and Lups (1929). The system of lumina, in a sagittal section, has roughly the shape of an H because of these diverticula and the downward bend of the very top of the aboral lumen, and it has the same appearance in a horizontal section (fig. 47) because of the two dilatations with the narrow channel between them.

In the attempt to trace the origin of the different parts of glandular tissue from the different walls of Rathke's pouch the regular mode of proliferation facilitates the interpretation. The growing compact buds can be followed to the wall which gave rise to them, at least in the earlier stages before a large compact body is formed. This is shown in fig. 48, in which the limiting membranes of the buds were made visible by Gömöry impregnation. I have thus been able to state which walls of the gland are derived from the different parts of Rathke's pouch, and I have indicated this in fig. 43. This is important, for now the definite shape of the gland is practically clear and the following changes cannot disturb this arrangement. It can be seen that the caudal part of the organ in the adult is formed by proliferation from the aboral lobe in the embryos and the rostral part of the gland

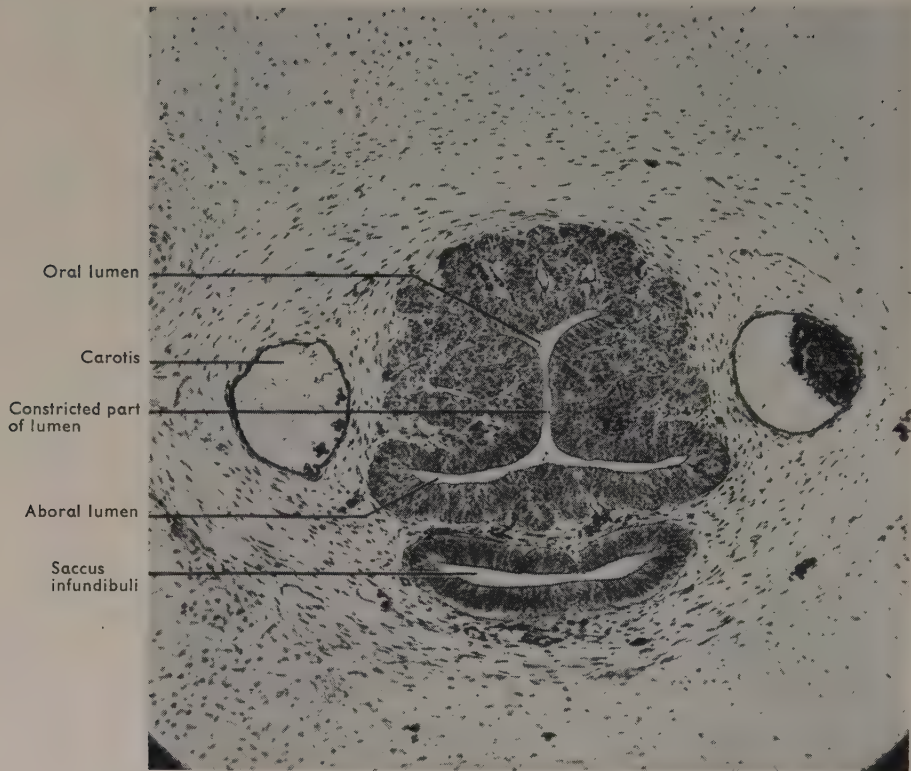


Fig. 47. *Larus ridibundus*, embryo CR 27 mm. Horizontal section through the pituitary, showing development of lumina. — Susa, paraffin 6μ , azan. $125\times$.

(cephalic lobe) is formed from the oral lobe and the anterior process. The constricted part of the anlage does not proliferate so rapidly and therefore the caudal lobe and the cephalic lobe in the older embryos are separated by a furrow. Such a bilobation of the anlage was observed also by Lups (1929) in the duck and the fowl, and he applies the terms of Woerdeman in approximately the same way as I have done above. The part called "Ventersäckchen" in his fig. 13 corresponds to the region in the gulls where the epithelial stalk is continuous with the body of the gland. I have observed a more or less distinct diverticulum from the caudal wall of the oral lumen here in a few embryos, and this structure might perhaps be called "Ventersäckchen" and be compared to the corresponding structure in *Selachii*, but I regard this diverticulum as too inconstant and too variable to be interpreted with certainty.

Embryo 22 mm—adult. The changes during this period are not very radical. The epithelial stalk degenerates slowly. It is discontinuous already in an embryo of 27 mm CR-length, and in most embryos measuring 80—100 mm TL it is absent or fragmentary. However, it was continuous even in one hatching young (TL about

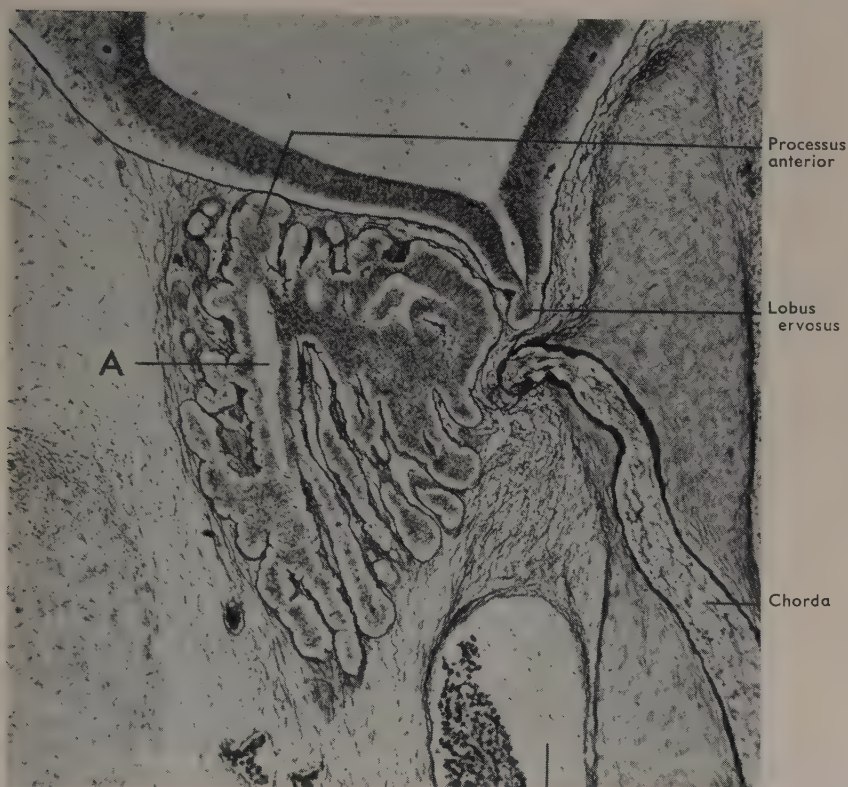


Fig. 48. *Larus ridibundus*, embryo CR 18 mm. Median section impregnated according to Gömöry to show the regular mode of proliferation. The compact buds are surrounded by reticular membranes which become distinct after impregnation. — Susa, paraffin 8 μ , silver impregnation according to Gömöry. 105 $\times$.

100 mm) so the variation is considerable. The degeneration of the stalk is characterized by the appearance of large cysts in the epithelium (cf. fig. 45), and these cysts often grow to a size several times the diameter of the stalk. The cysts contain a fluid which seems to contain little stainable proteins for it is usually very light blue in the azan preparations. The residual lumen of the gland may be absent in 30–40 mm embryos (TL c:a 50–70 mm), but it may also be present in embryos ready to hatch, so the variation is great also in this respect. Especially the aboral lumen is often preserved until late stages, as a rule in the form of a bladder near the neural lobe. The intermedia, that is the wall next to the neural lobe, is still single-layered in one 20-mm embryo, but in another it has lost contact with the neural lobe and begun to proliferate, and this has occurred in all older embryos. The lateral lobes (pars tuberalis) lose their lumen more or less rapidly also in their distal parts and give rise to numerous cords which cover the surface of the diencephalon. Yet the pars tuberalis has not reached its full extension in any unhatched specimen. Thus, for instance, I have never seen it form a complete

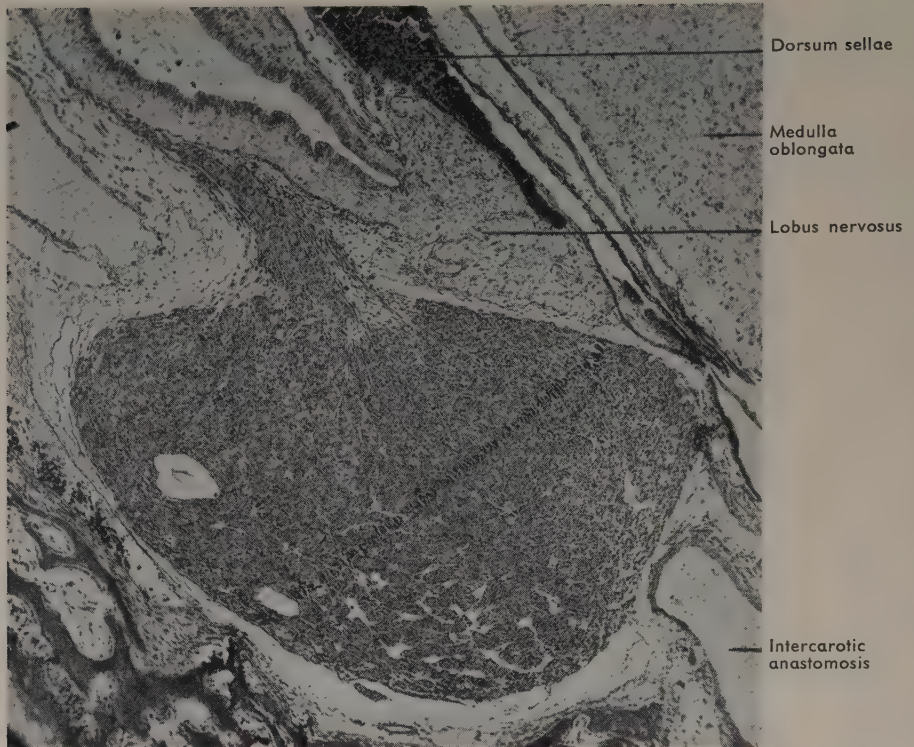


Fig. 49. *Larus ridibundus*, embryo CR 50 mm, TL 96 mm. Median section showing approximately adult conditions in the pituitary. The pars tuberalis (portal zone) is well developed. — Susa, paraffin 4 μ , azan. 85 $\times$.

collar around the infundibular stem in embryos, so that the fusion of the lateral lobes around the infundibular stem occurs during the post-embryonic development. The lateral lobes which originally are widely separated and reach the surface of the brain independently of each other, fuse in the mid-line between the pars distalis and the eminentia mediana and form the portal zone. This also occurs after the embryo has hatched, for in young just breaking the egg-shell the two partes tuberales are still separate, and even in adult gulls it is sometimes possible to trace the double origin of the portal zone.

A very important feature in the pituitary development in *Larus ridibundus* is the slow disappearance of the epithelial stalk and the lumina. This makes it possible to state definitely that the morphological relations in the 20-mm embryos remain practically unaltered till the adult stage. In fig. 43 G I have drawn the pituitary of a young of 28 cm total length. Here we still have distinct proximal and distal lumina left, and the epithelial stalk is recognizable. Three fixed points are thus available for direct comparison with younger stages, and in addition the origin of the pars tuberalis and the relation to the neural lobe gives some support to the interpretation.

Riparia riparia

In the sand martin the morphogenesis of the pituitary is much more rapid than in the black-headed gull. The embryonic structures such as epithelial stalk and residual lumen disappear very early and it is therefore not easy, as in the gull, to map out the adult gland in relation to the different parts of Rathke's pouch.

Embryos 3.5—5.2 mm. In the 3.5 mm embryo (with 9 pairs of somites) there is no indication of Rathke's pouch, the medullary tube is not closed rostrally. In the next embryo (4 mm, 20—21 pairs of somites) the oral groove is deep and the Rathke's pouch is recognizable though not well defined in the median section. No distinct praemandibular cavities can be detected. In an embryo of approximately the same stage (5 mm, 20—21 somites) the same can be observed but here also small cleft-like praemandibular cavities are present. They are connected by a string of mesenchyma just above Rathke's pouch. In an embryo with 29 pairs of somites (5.2 mm) the Rathke's pouch is somewhat more distinct though still with a wide opening and there is a slight invagination also behind the oral membrane marking the origin of Seessel's pouch. The praemandibular cavities are large and distinct here and the transversal connection between them is a distinct string of mesenchyma. The top of the notochord is connected with this transversal string by a row of cells (fig. 50). In the most advanced of these embryos (5 mm) the caudal somites have largely disintegrated, but the praemandibular cavities are distinct. Here the oral membrane has partly disappeared, but some remnants of it still make it possible to identify the shallow Seessel's pouch (fig. 51). The Rathke's pouch has been narrowed and more tube-shaped, and near the base there is a distinct evagination on each side: the lateral lobes. They are still only low ridges on the pouch.

Embryos 7—9.7 mm. In all these embryos the praemandibular cavities have disintegrated, and the oral membrane has disappeared. The Rathke's pouch has grown out, and a slender epithelial stalk is formed. Also the Seessel's pouch has proliferated considerably in all these embryos, and has formed a long, compact bud (*Gemma della tasca di Seessel* of Bruni 1914). This bud is directed more caudally than the Rathke's pouch, and the top of it reaches an area distinctly behind the intercarotic anastomosis which has been formed in these embryos (fig. 52). In contrast to the conditions in the gull the Seessel's pouch arises independently of the Rathke's pouch from the oral epithelium, and there is no distinct Vestibulo-pharyngo-ipofisario. It is thus indisputable that Seessel's pouch has nothing to do with the formation of the true pituitary anlage — it has not even been included in the epithelial stalk at this stage. Further, if the epithelial bud from it disintegrates, its disintegration products can hardly contribute to Rathke's pouch, for it is separated from the latter by the carotic anastomosis.

Even in the least advanced of these embryos the aboral parts of the Rathke's pouch are bent caudally, and this bend is probably caused by the dislocation of the infundibulum as in *Larus*. The lateral lobes have grown up to the brain on each side

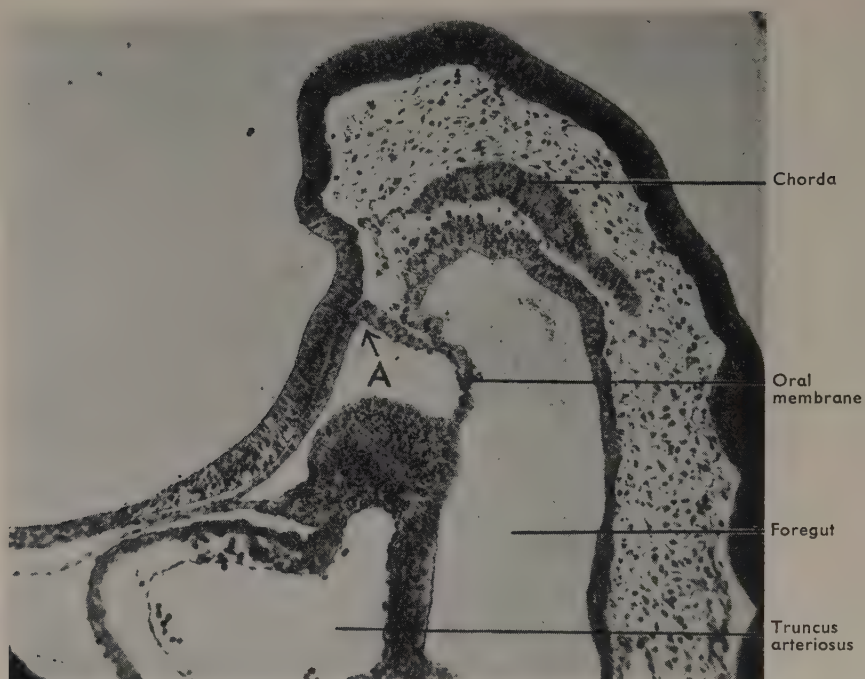


Fig. 50. *Riparia riparia*, embryo CR 5.2 mm, about 29 pairs of somites. Median section, showing first appearance of Rathke's pouch (A) and its relation to the oral membrane. — Bouin, paraffin 8μ , azan. $115\times$.

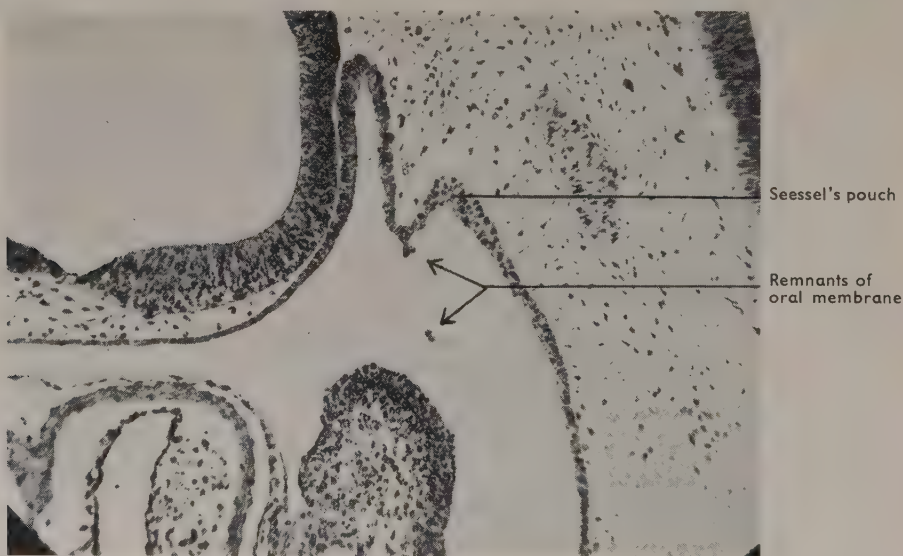


Fig. 51. *Riparia riparia*, embryo CR 5.0 mm. Median section showing the origin of Seessel's pouch. As small remnants of the oral membrane are left the entodermal origin of this pouch is quite distinct. — Bouin, paraffin 6μ , haematoxylin of Ehrlich and eosin. $115\times$.

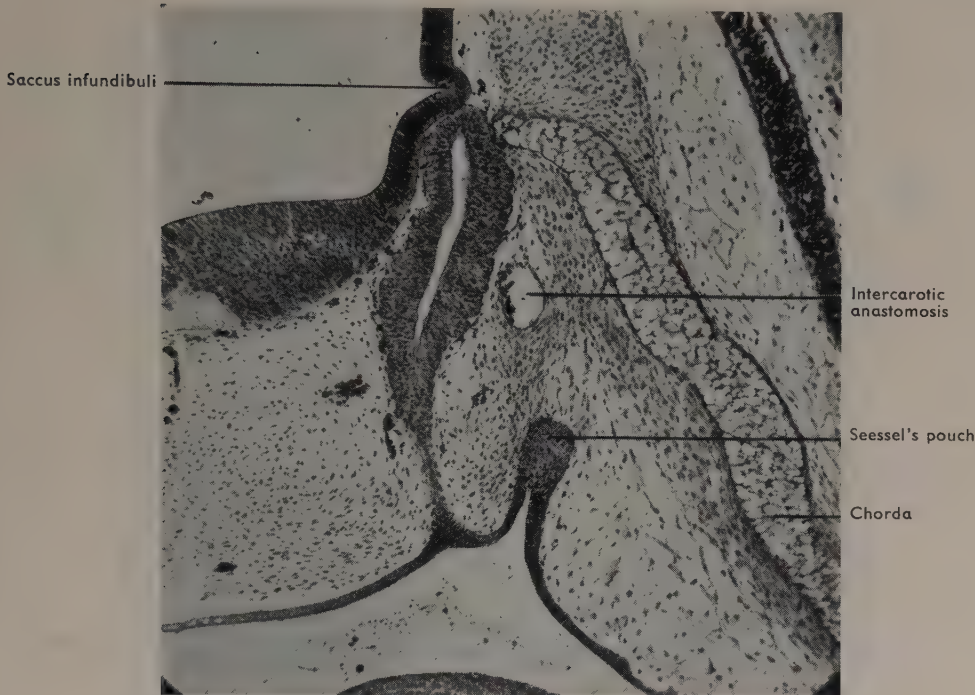


Fig. 52. *Riparia riparia*, embryo CR 7.8 mm. Median section, showing Rathke's pouch, Seessel's pouch, intercarotic anastomosis and notochord. A compact "gemma della tasca di Seessel" has been formed from Seessel's pouch and has a direction which is caudal to the intercarotic anastomosis. — Susa, paraffin 8 μ , iron haematoxylin of Heidenhain. 115 $\times$.

of the pouch and, in the most advanced of these embryos, established an intimate contact with it and begun to proliferate. The lumen of the Rathke's pouch has disappeared in the epithelial stalk, and in some embryos also in the proximal part of the lateral lobes. The lumen in the central parts of the anlage is, however, preserved in all these embryos. It presents a slight constriction from the sides which separates two dilatations as in *Larus*. The aboral lumen is never very broad but the oral one is more like that of the gull. The latter is connected with the lumina of the lateral lobes. All walls produce numerous compact buds and proliferate except the one in contact with the neurohypophysis. This "intermedia" is slow in proliferation and is single-layered in two of these embryos, but in the other it has begun to proliferate and has been separated from the neural lobe by mesenchyma. We have thus the same fixed points for identification of the different parts of the anlage as in the gull: the epithelial stalk origin, the two lumina with the constriction between them, the "intermedia", and the points of origin of the lobi laterales (fig. 53).

Embryos 10.0 mm—adult. In all specimens from the 10-mm stage on the epithelial stalk is absent, or, there are some small remnants of it near the oral ectoderm. The disappearance of the stalk in this species is thus much more rapid and complete

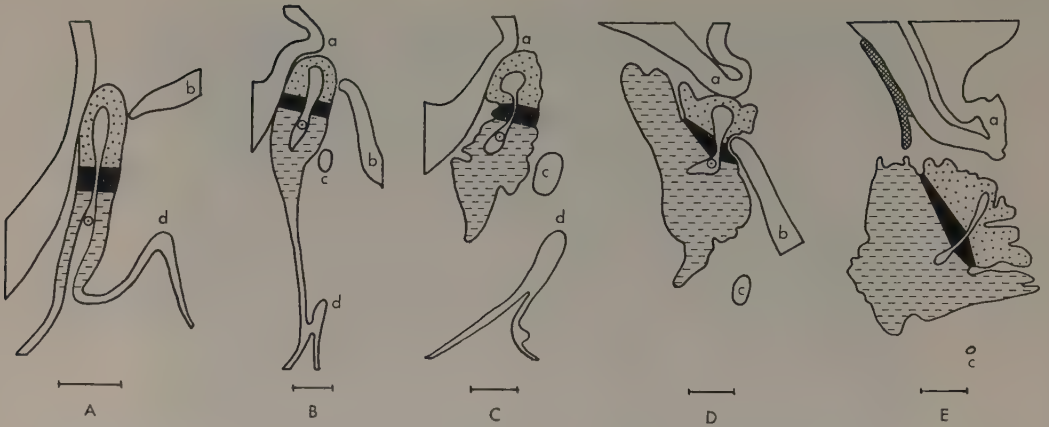


Fig. 53. *Riparia riparia*. Drawings of median sections showing the morphogenesis of the adenohypophysis. Stippled = aboral lobe, short lines = oral lobe, black = part derived from the constricted part of the pouch, crossed lines = pars tuberalis. a = saccus infundibuli, b = notochord, c = intercarotic anastomosis, d = Seessel's pouch.

A = CR 6 mm, B = CR 7.8 mm, C = CR 8.5 mm, D = CR 10.5 mm, E = CR 19 mm (hatching stage).

The black area in E could not be perfectly delimited because of the rich proliferation. The magnification is given by the line under each figure, representing 0.1 mm.

than in *Larus*. No trace of the Seessel's pouch has been observed, except in one 15 mm embryo, where it may correspond to a few cell cords near the oral ectoderm. It seems probable that Seessel's pouch as well as the large bud formed by it are withdrawn and the cells are included in the pharyngeal epithelium again. Though I have many embryos of the critical age (7–10 mm, cf. table 2) I have never seen the epithelial bud disintegrate or degenerate, and I think it little probable that such a process should occur so rapidly that none of the embryos would show it. This theory of withdrawal of the bud is based only on the fact that no other explanation seems satisfactory. Sympathetic ganglia appear also in this species in the region of the carotic anastomosis, but they have certainly nothing to do with Seessel's pouch. If they did come from this structure it would be possible to observe that cell masses emigrated from it.

The proliferation of the hypophyseal anlage which began in the preceding stages continues. The lumen is still partly left in the 9 embryos of 10–12 mm CR-length, and this is important for it allows us to understand the structure of the gland better. The aboral lumen in these embryos is fairly wide from side to side. The constriction is also recognizable, and separates it from the oral lumen. The latter does not show any diverticulum corresponding to the "Vorraum" in *Larus*, but there is an extensive proliferation from this surface (fig. 54). The "intermedia" may in some embryos of this size still be single-layered, but in older embryos it has invariably begun to proliferate. Also the lateral lobes has given rise to several buds which begin to cover the brain.

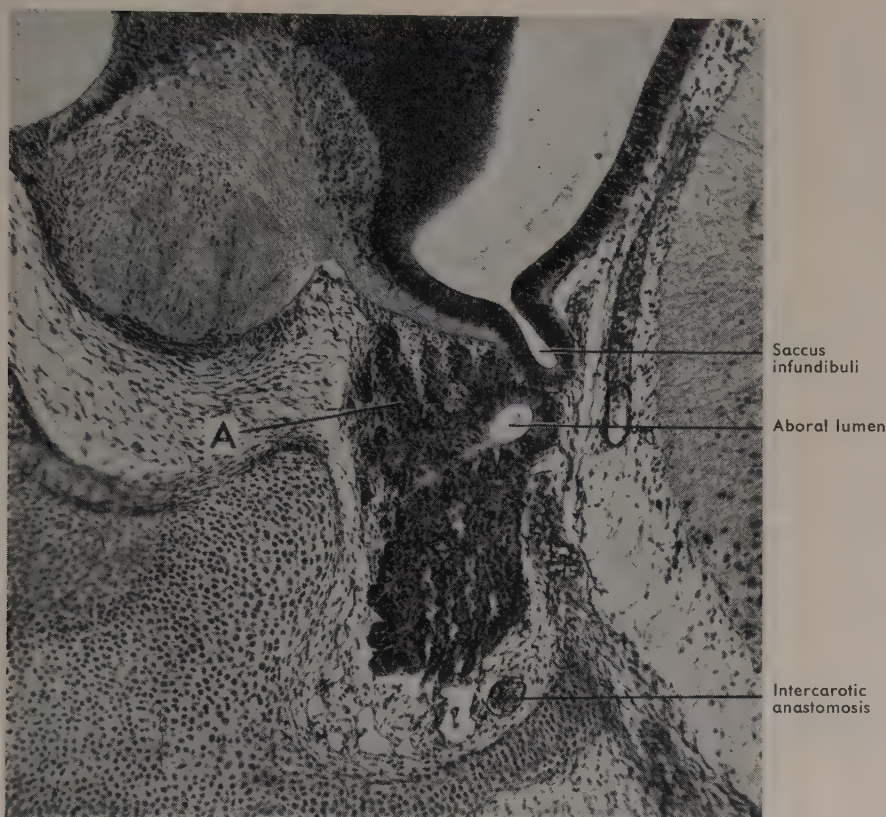


Fig. 54. *Riparia riparia*, embryo CR 12 mm. Median section, showing remnants of the lumen of Rathke's pouch and the zone of intensive proliferation corresponding to the processus anterior (A). — Bouin, paraffin 8μ , haematoxylin of Ehrlich and eosin. $100\times$.

After the 12 mm stage the transformations are not very radical. The proliferation continues, and the lumen disappears sooner or later. There is considerable variation as to the time of persistence of the residual cleft, thus it is quite absent in one embryo measuring 16 mm but is left as a small bladder in a young just after hatching.

The pars tuberalis, in old embryos, is fairly like that of the adult, but no fusion of the epithelial anlagen from each side behind the infundibular stem takes place before hatching. It is not even complete in a young of 43 mm total length, but in the adult there is such a connection. The fusion of the two anlagen forming the unpaired portal zone is, however, completed just before hatching. The pars tuberalis is relatively much better developed in embryos than in adult birds and young, where it mainly consists of single-layered plates or scattered strings of cells. On the whole it seems to be retarded in its development during the later part of ontogeny.

Fig. 53 shows that it is possible to make a diagram also for *Riparia* showing the relations between different parts of Rathke's pouch and the structure of the adult gland. The details are, however, not so exact here, because the system of lumina etc. are reduced very early. The changes which may occur from the 12 mm stage, when the details still can be mapped out exactly, to the adult stage are, however, slight.

Gallus gallus

For most details I refer to Bruni (1914), Atwell and Sitler (1918), Atwell (1939) and Rahn (1939), and their records will only be commented on here together with a few comparisons with the preceding species. It may be stated already now that the fowl is a favourable object, for also here the original structures of Rathke's pocket are preserved for a long time.

Early stages. As stated by Atwell and Sitler (1918) and Lillie (1930) the Rathke's pouch appears in the chick embryo at the 20-somite stage approximately. In my preparations there is no pouch in the 12 and 17-somite embryos, but it is distinct in the three embryos with 23—24 pairs of somites. The lateral lobes appear very early as lateral ridges on the base of the pocket, according to Atwell and Sitler after 59 hours of incubation. I found distinct lobuli laterales in my 72-hour embryos. In these stages the Seessel's pouch is wide and shallow, and closely attached to the caudal surface of Rathke's pouch. The indention of the two invaginations is quite clear in these embryos, for the oral membrane is still present. I have found distinct remnants of this membrane also in one of my 72-hour embryos.

Embryos 3—3.5 days. The Rathke's pouch is deep and distinct, closely attached to the brain wall. The lateral lobes are clearly visible in three embryos but less distinct in the fourth. The praemandibular cavities are distinct though small in one of the specimens, and have no relation to the lateral lobes. The wide pocket of Seessel is closely attached to Rathke's pouch in all the specimens, but in the 3.5-day embryo the lumina of the pouches communicate directly through a large aperture in the ento-ectodermal wall. Probably this has no morphological significance beyond the fact that it is an expression for the obliteration of the separating wall.

Embryos 4—5 days (fig. 55). The changes taking place in these embryos are: 1) A constriction of the basal parts of Rathke's pouch so that an epithelial stalk is formed. 2) Further growth of the lateral lobes so that they are in contact with the brain in the oldest embryos. 3) A process projects from the rostral side of Rathke's pouch between the bases of the lobuli laterales. This anterior process contains a lumen, the anterior diverticulum, in some of my embryos, but it was compact in the 5-day embryo described by Atwell and Sitler (1918). 4) Regression of Seessel's pouch and formation of a solid bud from its epithelium. There is a deep "Vestibulo pharyngo-ipofisario" from which both pouches originate, probably formed by obliteration of the ento-ectodermal wall between the pouches. 5) Proliferation has begun from the walls of Rathke's pouch but not from the one attached to the infundibular process ("intermedia"). 6) The aboral half of Rathke's pouch,

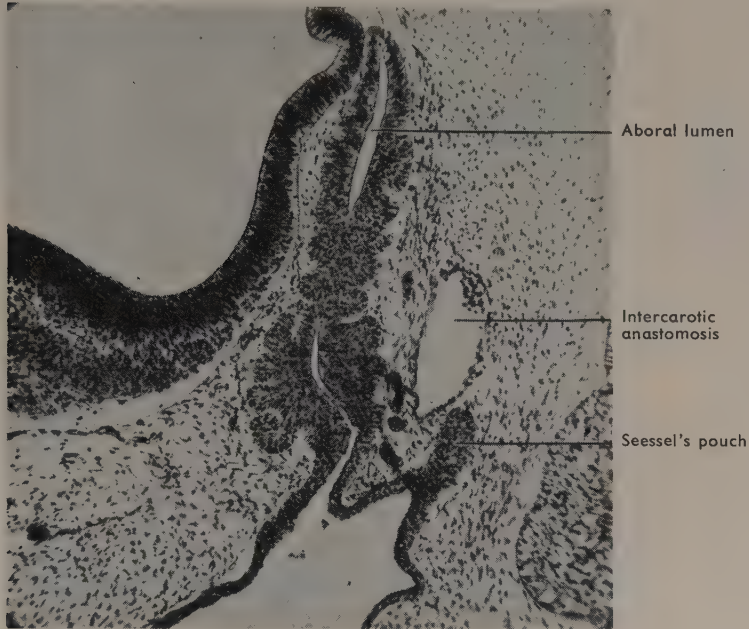


Fig. 55. *Gallus gallus*, embryo 5 d. Paramedian section. Note the compact bud from Seessel's pouch and its direction in relation to the carotis. The lateral parts of the oral and aboral lumina are visible but the constricted part of the lumen is outside the section. — Bouin, paraffin 8 μ , azan. 100 $\times$.

in the older of these embryos, is bent caudally, and the point of the angle corresponds approximately to the point where the anlage meets the brain, just above the bases of the lateral lobes. The lumen is narrowed from the sides near the point of the angle, so aboral and oral lumina are formed.

Some other hollow buds are formed by the pocket, but they do not seem to be very constant. In two of the embryos there is a distinct hollow process from the posterior surface of Rathke's pouch. Whether this diverticula is homologous with the "Ventrialsäckchen" in Selachii, perhaps also in reptiles, described by Woerdenman (1914) seems uncertain because of its variability. Such a process has, however, been seen in the duck (Lups 1929) and in the pigeon (Rost 1939).

It may be noticed also that in one five-day embryo there is a distinct cellular bridge from the compact Seessel's pouch to the posterior wall of Rathke's pouch, probably as a consequence of a previous contact between the walls. St Remy's interpretation of similar structures has been commented above (p. 81).

Embryos 6—7 days (fig. 56—57). This stage will be considered more in detail since it is now possible to define the different parts of the pouch, but it should be mentioned that there is some variation among the specimens of this age.

The hypophyseal anlage is now bent at an approximately right angle. The lumen is narrowed near the base of the pouch, where an epithelial stalk is being formed,

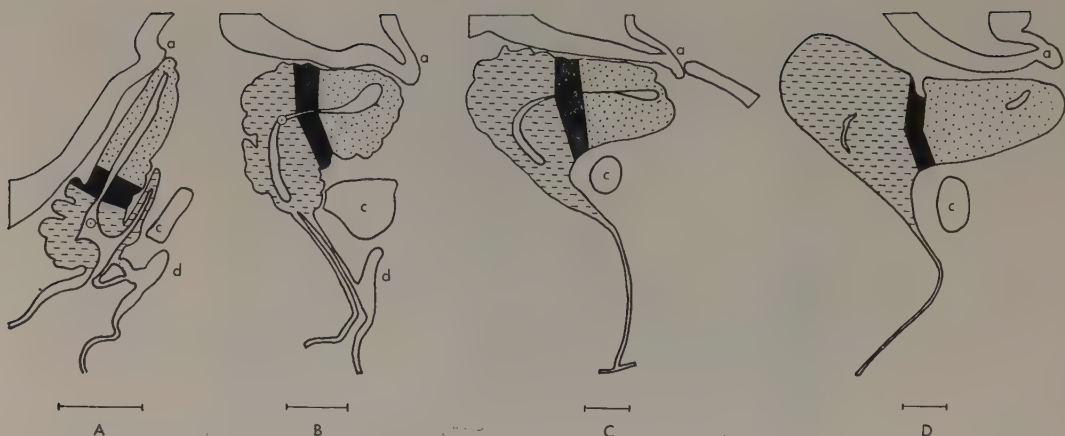


Fig. 56. *Gallus gallus*. Drawings of median sections showing the morphogenesis of the adeno-hypophysis. For explanation see under fig. 53 (p. 90).

Time of incubation: A = 5 d, B = 7 d, C = 11 d, D = 13 d.

Note the bridge of tissue between Seessel's pouch and Rathke's pouch in fig. A. The delimitation of the black area in fig. D is not quite certain for reasons mentioned under fig. 53 and 43. The stalk has been completed from adjacent sections in fig. B—D. The line under each figure represents 0.5 mm.

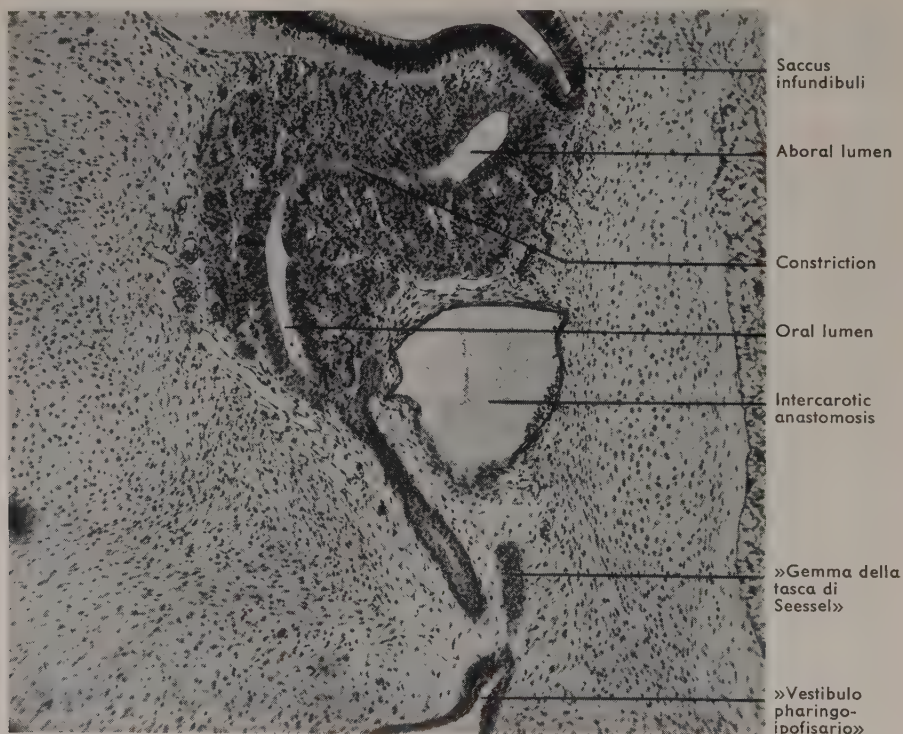


Fig. 57. *Gallus gallus*, embryo 7 d. Median section showing the bipartition of the lumen, and the caudal bend of Rathke's pouch. Note also the compact bud from Seessel's pouch and its relation to the carotis. — Bouin, paraffin 8μ , azan. $93\times$.

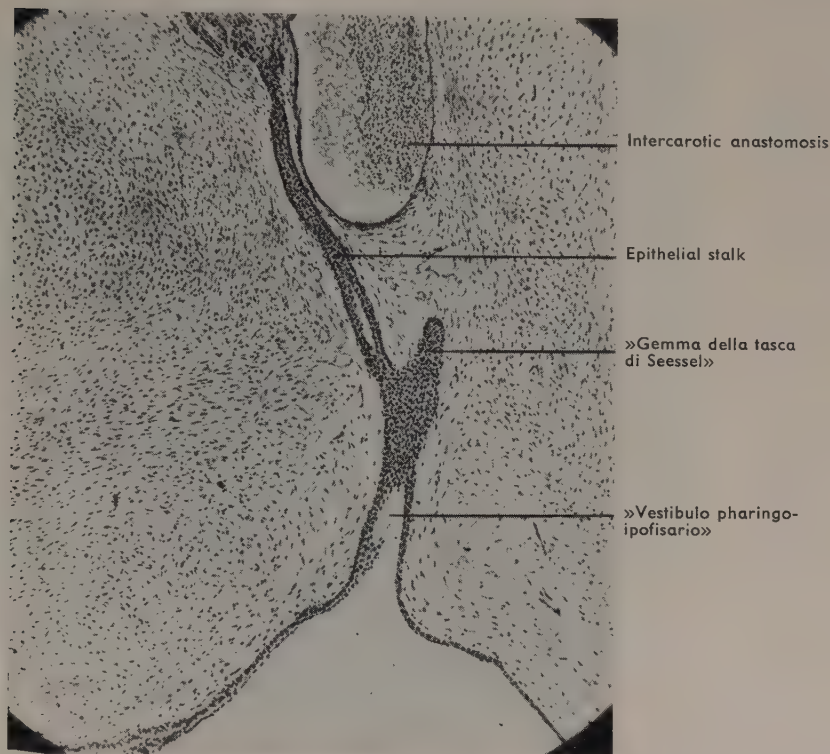


Fig. 58. *Gallus gallus*, embryo 6 d. Median section showing the epithelial stalk and the position of Seessel's pouch. — Alcohol-formalin-acetic acid, paraffin 8 μ , Bodian. 80 $\times$.

and there is also a distinct constriction between the oral and aboral lobes. Both these lobes contain lumina which are broad from side to side, the oral one receiving the lumina which are contained in the lateral lobes. In most embryos there is a distinct anterior process from the rostral side of the oral lobe. Even if its lumen, the anterior diverticulum, is sometimes absent or indistinct there is always a zone of extensive proliferation at the same place. Also a median posterior diverticulum is sometimes present, but like some other hollow processes which sometimes appear it is not constant.

The walls of the pouch and the lateral lobes have begun to proliferate, but the intermedia is still single-layered. The area of contact with the brain has been reduced to the very tip of the pouch which is still in contact with the infundibular process. This separation is caused by the invasion of mesenchyma between the brain and the pouch already in the 5-day embryos.

The lateral lobes have also begun to proliferate and still contain a lumen though this is sometimes indistinct in the basal parts of the processes.

Seessel's pouch is reduced in these embryos, but it may still have a compact bud which is situated near the base of the epithelial stalk (fig. 58). It should be

mentioned that I have searched in vain for signs of disintegration of Seessel's pouch and its bud also in these embryos. Groups of ganglion cells are, however, commonly seen near the carotids, but I have found no evidence of a relation between them and Seessel's pouch.

The 6-day embryos of *Gallus* are thus directly comparable to embryos of *Larus* and *Riparia* as regards the differentiation of Rathke's pouch. We have the same fixed points which allow us to follow the further differentiation in detail: lateral lobes, oral and aboral lumina and the constriction between them, epithelial stalk, and "intermedia". There is also an external constriction of the anlage corresponding to that of the lumen. I admit, however, that the external constriction between oral and aboral lobes may be less distinct, especially after it has been filled out by strings from the proliferating lateral lobes. Rahn (1939) believed that he could find distinct transversal furrows on the dorsal and ventral sides of the anlage which separated the aboral (caudal) and oral (cephalic) lobes in the 6-days chick. Though I am convinced that Rahn was right in his main conclusions concerning the development of these lobes I must agree with Payne (1942) who found these furrows to be excessively varying and little useful as a basis for comparisons.

Embryos 8 days—adult. The development of the pituitary from the 6th day to the adult stage has been described in detail by Rahn (1939) and Atwell (1939). The epithelial stalk has partly lost its lumen in some 6—7-days embryos and is later reduced. It is still continuous in some of my 11-days embryos but not in older specimens. It should be noted that the stalk here is a "pedunculo vestibulopofisario" according to Bruni's terminology (1914) for it is formed exclusively by Rathke's pouch. The "vestibulo pharingo-ipofisario" may in some cases disappear entirely, for the minute remnants of Seessel's pouch are sometimes quite separated from the epithelial stalk. In some cases it forms a small part near the base of the epithelial stalk, which then carries the remnants of Seessel's pouch as a small bud not far from the oral epithelium. In neither case can the entodermal pouch have anything to do with the formation of the pituitary.

The bend of the anlage which was distinct in 6-day embryos becomes still more pronounced, and in later stages the pouch is, in fact, C-shaped as seen in a median section. The lumen of the constricted part disappears at an early stage and also the oral lumen remains small. The aboral lumen, however, is present in all my embryos up to the 13th day of incubation. The "intermedia" retains its contact with the neural lobe up to this age approximately and remains single-layered whereas the other walls of the pouch proliferate. As soon as the contact is lost, the intermedia begins to proliferate and is included in the rest of the caudal lobe. The lobi laterales grow out and form the pars tuberalis, but the closure of the ring around the infundibular stalk is completed after hatching.

As will be seen in fig. 56 it is possible to map up the fowl pituitary in a similar way as for *Larus* and *Riparia*. Since the lumina disappear at a rather early stage the scheme is perhaps not so complete as that for *Larus* but the main features are indisputable (cf. also fig. 59).

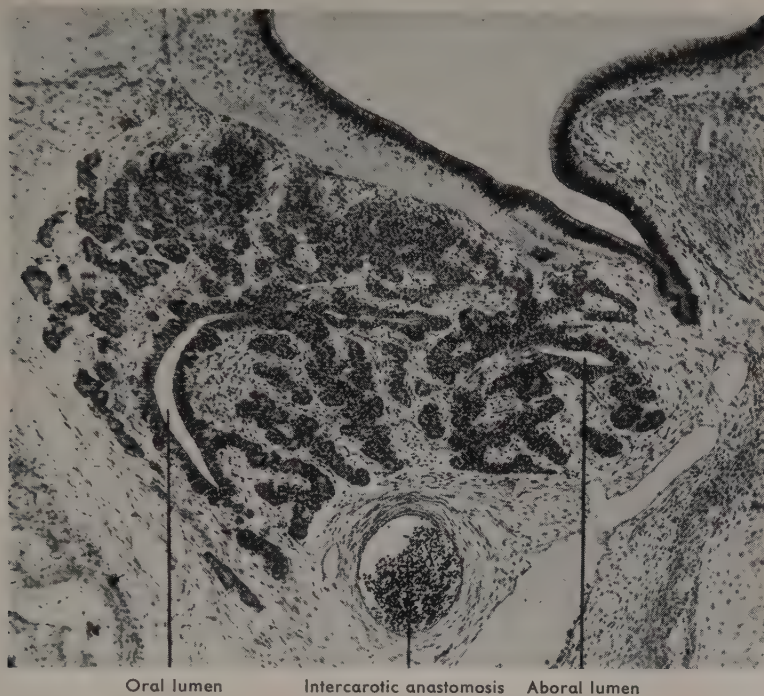


Fig. 59. *Gallus gallus*, embryo 11 d. Median section of the pituitary, which has attained approximately its final shape. The course and relationships of the lumen are still distinct. — Bouin, paraffin 8μ , azan. $110\times$.

Summary and Discussion of the Morphogenesis of the Adenohypophysis

1. The adenohypophysis is formed by Rathke's pouch, and a contribution from the entodermal Seessel's pouch could not be observed in the investigated species (*Larus*, *Riparia*, *Gallus*). Modern investigations indicate that the pituitary is purely ectodermal also in *Anas* (Friedman 1934) and *Columba* (Rost 1939).

2. At a certain stage of development (9—14 mm embryos in *Larus*, 7—9 mm embryos in *Riparia*, and 6 day embryos in *Gallus*) it is possible to distinguish the following homologous structures in the different species: 1) an aboral and an oral lobe and corresponding dilatations of the lumen with a constriction between them. 2) an epithelial stalk. 3) the lateral lobes, the lumina of which are continuous with the oral lumen. 4) a contact zone with the brain, which has been reduced to a small area between the top of the pouch and the growing infundibular process. This area remains single-layered to a fairly late stage of development whereas the other walls proliferate rapidly with the exception of the epithelial stalk. The single-layered wall corresponds morphologically to the intermedia of other vertebrates.

3. The adult gland is developed from this stage in the following way: 1) The caudal lobe in the adult is formed by proliferation from the aboral lobe of the

embryo (die Rathkesche Tasche of Woerdeman 1914), perhaps also by a few strings from the walls of the constricted part of the anlage. Also the "intermedia" loses its contact with the neural lobe, proliferates and is included in the caudal lobe. 2) The cephalic lobe in the adult is formed by the oral lobe and possibly by some strings from the constricted part. Massive proliferation takes place from the rostral (dorsal) side of the oral lobe, and in *Larus* and frequently in *Gallus* an anterior diverticulum is present here, corresponding to the Vorraum of Woerdeman. 3) The pars tuberalis is developed by proliferation of the lateral lobes which form the layers of epithelial tissue on the brain surface. In none of the species is the collar around the infundibular stem complete until after hatching. 4) The epithelial stalk is reduced, most rapidly and completely in *Riparia*. In *Larus* and *Gallus* the degeneration is characterized by the formation of cysts.

The comparison with the histological regions of the adult (caudal and cephalic lobes) here is of course only approximate. There is, however, a relation between the histological regions and the ontogenetic development. The details are discussed in the next part of the chapter.

Undoubtedly, the course of development of the adenohypophysis described here for *Larus*, *Riparia* and *Gallus* agrees well with that in *Anas*, described by Lups (1929) and Painter (1942). Lups made a detailed examination of the earlier stages and introduced Woerdeman's terminology, so direct comparisons can be made with my descriptions above. That the later part of the development in the duck is the same as in my species is clear from the beautiful microphotographs and descriptions of Painter. Rost's (1939) paper on the development in *Columba livia* is extremely detailed for the earliest stages, and the later development is treated more briefly. In his figure 7 he has drawn a stage, however, where it is possible to distinguish both lateral lobes, an aboral lumen (at RT), a constriction (at S₃) and an oral lumen. The latter has one anterior bud which is directly comparable to the anterior process, and one posterior bud. The anlage is bent slightly caudally. His figure of the oldest stage (Abb. 9) tends to show that the gland is differentiated in a way very similar to that which I have described for *Larus*, *Riparia* and *Gallus*. The bases of the lateral lobes are distinct in the figure and give at least one fixed point for comparison.

Histogenesis

Literature

The literature on the appearance and development of the active types of cells in the avian pituitary is somewhat confusing. The divergencies are probably due to the difficulty to define clearly the types of avian pituitary cells in general, but also to differences in the technique used.

The first to try a histogenetical study on the avian adenohypophysis was Schooley (1937, reprinted in Schooley and Riddle, 1938). Working with embryos

of *Columba livia* he found the first granulated cells in the adenohypophysis between the 12th and 16th day of incubation. These cells were said to be acidophilic and were the only differentiated cells found in embryos. In the full-term embryo (17—18 days) some cells with a “metabolic centre” similar to that of basophils were observed, but the cytoplasm was still ungranulated so they were classified as chromophobes. Granules did not appear in the basophils until the young were one and a half months old. The present investigation makes it probable that another technique may reveal differentiated cells at an earlier stage also in the pigeons. Studitsky (1940) found chromophilic cells in 10—11-day embryos of *Columba*.

Voigt (1939) studied the histogenesis of the fowl pituitary. He found a few basophils in 12-day embryos, but he admits that they may occur between the 10th and 12th day of incubation. As he could not distinguish them clearly from connective tissue cells (!) he could not make any definite statements as to their first occurrence. The basophils are said to appear throughout the gland at once, and belong to a “merocrine” type. A second type, characterized by holocrine function, is said to appear about 14 days after hatching and to attain full differentiation in animals about 3 months old. The first acidophils were observed in the rostral end of the gland of 11-day embryos. In older embryos the acidophils successively spread caudally whereas the older of them develop into a “mature” stage. In chickens 5 days after hatching even the caudal end had developed acidophils. When proceeding from this end in a rostral direction over the gland Voigt could find successive areas with more and more differentiated acidophils until the series was finished by chromophobic “Kernhaufen” at the very rostral pole. These chromophobe nuclear heaps were regarded as the final stage of the secretory cycle of the apocrine acidophils. Later new acidophils of this type appear more irregularly in the gland. Voigt also describes a type of holocrine acidophils in the young male.

The cellular differentiation in the fowl pituitary was also treated by Rahn (1939). He found a general basophilic tendency of nuclei and plasm in the cells of 8—9-day embryos. These cells were regarded as transitional cells between embryonic cells and chromophobes, not as true basophils. Unquestionable acidophils and basophils were found in 10- and 11-day embryos. The basophils were present throughout the gland and the acidophils were restricted to the rostral tip just as was described by Voigt, and also Rahn could observe the spread of acidophilic differentiation in a caudal direction. The acidophils of the caudal lobe appeared on the 18th day. By this time the basophils are said to be very few and indistinct. The pituitary of the newly-hatched chick is characterized by strong acidophilia and the basophils and chromophobes are few and poorly differentiated. The A_2 -cells are said to appear in the cephalic lobe after hatching. Fully differentiated basophils were not observed until the onset of sexual maturity. Similar results were obtained by Venzke (1942).

Payne (1942) makes some remarks on the histogenesis of the fowl pituitary. In chicks three days after hatching he could find only acidophils and chromophobes, and the basophils were distinct for the first time in ten day chicks. He found the A_2 -cells in the cephalic lobe as early as the 3rd day after hatching.

Painter (1942) used embryos of ducks for his investigation and was able to confirm most of Rahn's statements. In embryos incubated for 13—15 days he found a basophilic reaction of some nuclei, and the first true basophils and acidophils occurred in the 16-day embryos. Both kinds of cells were restricted to the cephalic lobe of the gland. The acidophils then spread over the gland, whereas the basophils disappear about the 19th day. The acidophils of the caudal lobe (A_1 -cells) become distinct in ducklings more than one week old. The A_2 -cells were identified in the cephalic lobe during the 2nd week after hatching, and by this time the basophils reappear in the cephalic lobe. Later some basophils occur also in the caudal lobe.

Own investigations. The results of the present investigations, including embryos of *Gallus*, *Larus* and *Riparia*, partly confirm the statements of Rahn and Painter. However, the introduction of the Bodian technique revealed some structures important for a correct interpretation of the histogenesis, and made some parts of the picture more complete.

Gallus gallus

First appearance of cellular differentiation: the Ag-cells. Voigt's and Rahn's statements to the fact that distinctly granular cells are found for the first time in the pituitaries of 10- or 11-day fowl embryos treated according to common methods is confirmed by the present investigation. I have also noted the faint basophilic reaction of some cells which becomes visible in still younger embryos and was reported by Rahn. The Bodian technique, however, made it possible to find distinctly granular cells from the beginning of the 6th day of incubation.

These first differentiated cells (Ag-cells) are characterized by the presence in their plasm of granules which readily reduce silver and thus can be demonstrated invariably by the Bodian technique (fig. 60). The granules become quite black, whereas the surroundings remain unstained or assume a very pale yellow or reddish tinge. The identification of the granular cells is thus no matter of subjective judgment — it is an objective registration of unmistakable and clear pictures.

First the significance of these granules should be discussed. As they are abundant in some cells and completely absent from other cells it may be safe to state that the cells differ in their cytochemical properties: in other words, a differentiation has taken place. The differences do not depend on changes which characterize the mitotic and growth cycles of embryonic cells in general, because no granules have been found in other growing epithelial structures in the preparations, such as epithelial stalk, oral epithelium, oesophageal epithelium, and epidermis. The Ag-cells are thus highly specific for the hypophyseal anlage; in fact, they are found nowhere else in the young embryos. In older embryos I have found similar cells in the stratum corneum of the epidermis only, but not in the more normal epithelial organs of the head. The only reasonable interpretation of the Ag-cells is that they

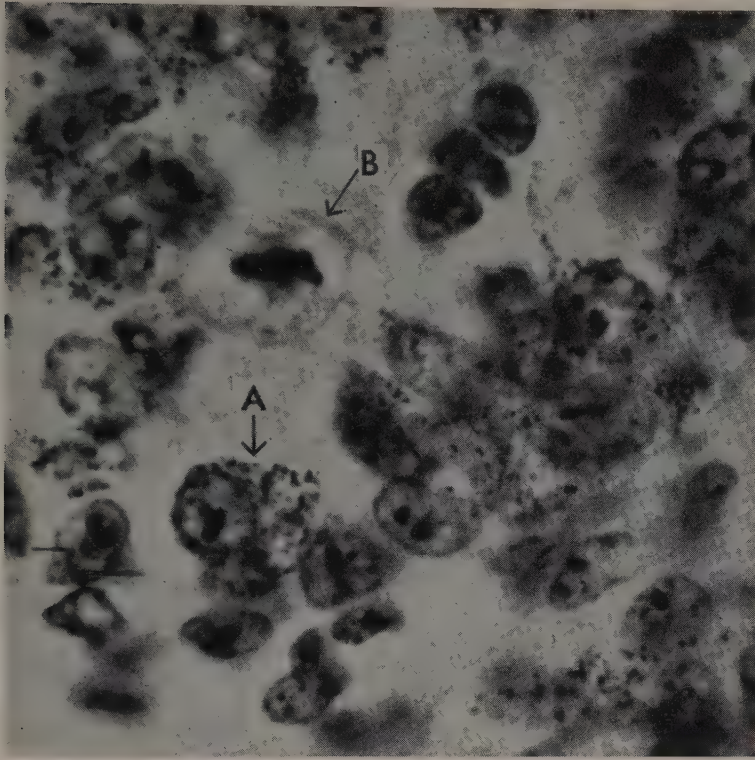


Fig. 60. *Gallus gallus*, embryo 6 d. Part of the oral lobe in a section impregnated according to Bodian. The differentiation of the glandular cells is distinct. In addition to non-granular cells there are numerous Ag-cells, one of which (A) is exactly in focus. B = metaphase. — Bouin, paraffin 8 μ , Bodian, 2000 $\times$.

are correlated with the specific function of the pituitary — i. e. the secretion of hormones.

Even if it is admitted that the reaction of the Ag-cells to silver solutions is significant and indicates a functional differentiation, it might be suggested that the granules themselves are artefacts. This is, however, contradicted by several observations. Thus, the granules are present in the same frequency and size even if different fixation methods are used. Thus, in a series of 6-day embryos, fixed in Carnoy, formalin, Bouin, and alcohol-formalin-acetic acid resp. I could find no differences in the granulations apart from those which are due to shrinkage of the cells in some fixatives. It is not probable that the granules should look identical after so different a treatment if they were the result of a precipitation process.

It might also be suggested that the argyrophilic granules are mitochondria, but their resistance to such fixatives as Bouin and Carnoy does not support this view. True mitochondria are destroyed by this treatment. Further, if they were mitochondria, the mitochondria would appear also in other cells in the same sections,

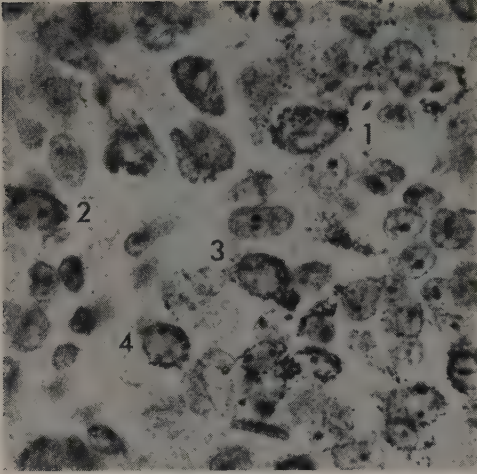


Fig. 61.

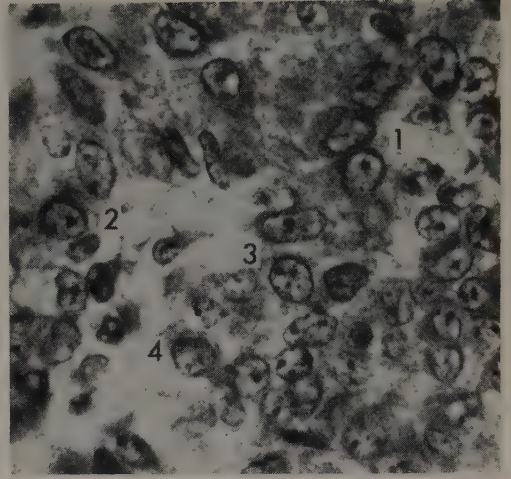


Fig. 62.

Fig. 61. *Gallus gallus*, embryo 10 d. Ag-cells in the oral lobe near the rostral end of the gland. — Bouin, paraffin 4 μ , Bodian. 1000 $\times$.

Fig. 62. The same area as in fig. 61 after the gold had been removed and the section had been stained in azan. It can be seen that some Ag-cells are characterized by an increased chromophilia, though it is not always distinct. The figures in corresponding places in the two photographs are intended to facilitate the comparison. Technique see fig. 61. Green filter. 1000 $\times$.

at least in older embryos. This is not the case. All these considerations lead to the conclusion that *the argyrophilic granules are present also in vivo, that they are not mitochondria, and that they constitute a mechanism engaged in the secretory function of the hypophyseal cells.*

The Ag-cells are recognizable also in thin azan preparations though they are so indistinct that they would certainly be overlooked if the observer did not know that they were there. In azan preparations they differ from the common embryonic cells by containing a slightly denser plasm, which appears to be a little darker than that of undifferentiated cells. In normally differentiated preparations they look bluish or violet but may change into reddish if the staining in azocarmine has been intensive. In some preparations fixed in Bouin it is even possible to distinguish red and bluish granules mixed in the plasm, but the blue component usually prevails (fig. 61—62). Probably, the slightly stainable cells observed by Rahn in young chick embryos and by Painter in duck embryos before the appearance of distinct chromophils were such Ag-cells. It should be noted, however, that Rahn observed these cells from the 8th day of incubation in the chick embryos whereas the Ag-cells definitely occur at the beginning of the 6th day. This discrepancy is explained by the difficulty to identify these cells after common staining, especially in the 6-day and 7-day embryos, whereas they become more distinct in older embryos.

The time of occurrence of the Ag-cells in *Gallus* has been fixed at the beginning of the 6th day of incubation (fig. 75). No such cells were found in 5-day and

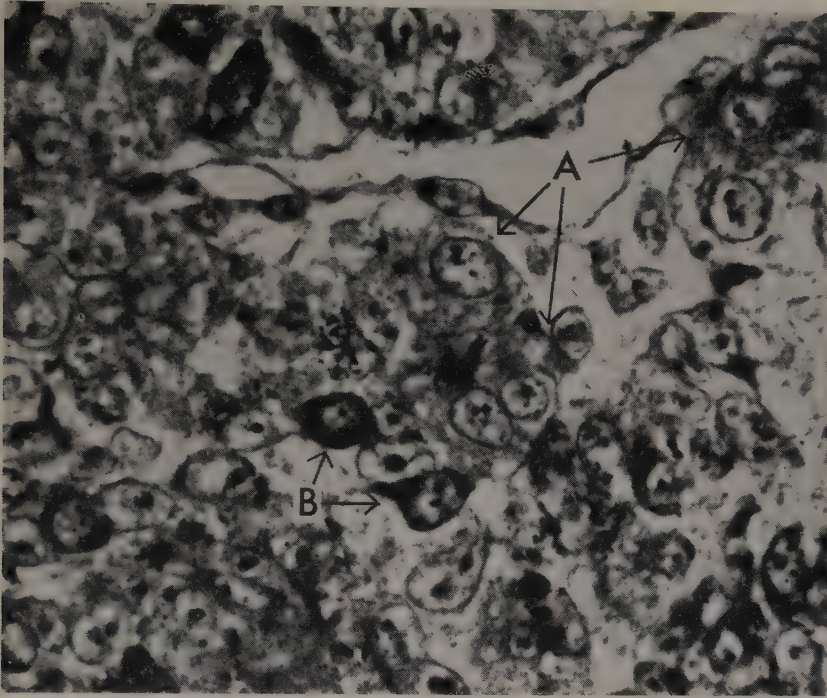


Fig. 63. *Gallus gallus*, embryo 9 d 6 hrs. Cells in the rostral end (oral lobe) of the pars distalis impregnated according to Bodian and then stained in azan without removal of gold. The black Ag-cells (B) can be readily distinguished and also some Ag-negative cells which contain chromophilic substance and therefore have taken some colour in the staining solutions (A). The plasma of the latter cells is, in the preparation, violet (neutral) because of the presence of both reddish and bluish inclusions. — Bouin, paraffin 4 μ , Bodian + azan. Yellow filter. 1400 $\times$.

younger embryos, but in an embryo of 5 days and 6 hours they were moderately numerous in the oral part of the gland. This specimen indicates that the differentiation starts from the rostral end but no definite conclusion can be drawn from a single observation. In the 6-day embryos the Ag-cells are present throughout the gland but are absent from the epithelial stalk, the "intermedia", and the top of the lateral lobes. It can be distinctly seen that the differentiation generally begins in the cells near the top of the epithelial buds, and that the cells adjacent to the residual lumen remain undifferentiated until later stages. This observation does not agree with Rahn's statement that the cells near the lumen should be the first to show signs of differentiation.

Further development of the Ag-cells up to the 10-day stage. In the 8-day embryos the number of Ag-cells has increased in comparison with the 6-day embryos, and certain regional regularities in their distribution have appeared. The Ag-cells are especially common in the caudal wall of the aboral lobe (fig. 64). If the gold is removed azan staining shows that the Ag-cells are more chromophilic than other

cells, but their affinity to stains is still slight. After intensive staining they contain both acidophilic and basophilic inclusions, and as the general colour therefore is intermediate between slightly basophilic and slightly acidophilic I call this transitional type of granulation "neutral". If Bodian impregnation is combined with azan staining it appears that a few such cells with neutral granulation lack argyrophilic granules (fig. 63). Thus the neutral granulation and the argyrophilic granules are not always coexistent although this is usually the case.

In the 9- and 10-day embryos the histological bipartition of the gland becomes distinct (fig. 64). The Ag-cells make up a very high percentage of the caudal (aboral) lobe, whereas they are relatively fewer in the cephalic (oral) lobe. Further the Ag-cells of the cephalic lobe tend to be more densely packed with granules whereas the granulation of the caudal lobe cells is more diffuse. The latter part of the gland therefore looks more uniform in the Bodian preparations. As the lumina are partly preserved at this stage it could be stated with certainty that the histological regions correspond to the oral and aboral lobes of the Rathke's pouch.

Appearance of chromophilic cells on the 11th day of incubation. The first distinct acidophils and basophils are present in my 10.5- and 11-day embryos, and both types seem to arise at the same time. Both kinds of chromophilic cells undoubtedly develop from slightly stainable cells of the "neutral" type described above, for every stage of transition between such cells and true basophils and acidophils can be found. The slightly stainable "neutral" cells are common in the 10- and 11-day embryos. Some of them contain Ag-granules, some of them do not (fig. 63).

In the 11-day embryos the acidophils are restricted to the very rostral tip of the pars distalis. They contain granules which stain distinctly red in azan, but they never contain any argyrophilic granules. In fact, I have never found any Ag-granules in cells which contained so much acidophilic matter that an identification was possible. As the youngest identifiable acidophils are only slightly different from the bleach "neutral" cells it may be concluded that the neutral cells without Ag-granules are the prospective acidophils of the cephalic lobe (A_2 -cells).

The basophils are also concentrated in the rostral end of the gland though not as distinctly as the acidophils. They contain a basophilic substance, in which granules are difficult to observe. The fully differentiated embryonic basophils are packed with argyrophilic granules and therefore look entirely black in Bodian preparations. They are connected with the slightly stainable "neutral" cells by a continuous series of transitional forms, which contain numerous Ag-granules throughout. It is therefore very probable that the "neutral" cells which contain argyrophilic granules are the prospective basophils.

The differentiation of the cephalic lobe cells into basophils and acidophils is apparently prepared in the younger embryos, in which some cells, the prospective basophils, acquire argyrophilic granulation, whereas most cells, the prospective acidophils, do not. The origin of the two types of cells from Ag-cells and ungranulated cells respectively is also supported by the numerical conditions. The Ag-negative cells are by far the predominant type in the cephalic lobe of 9- and

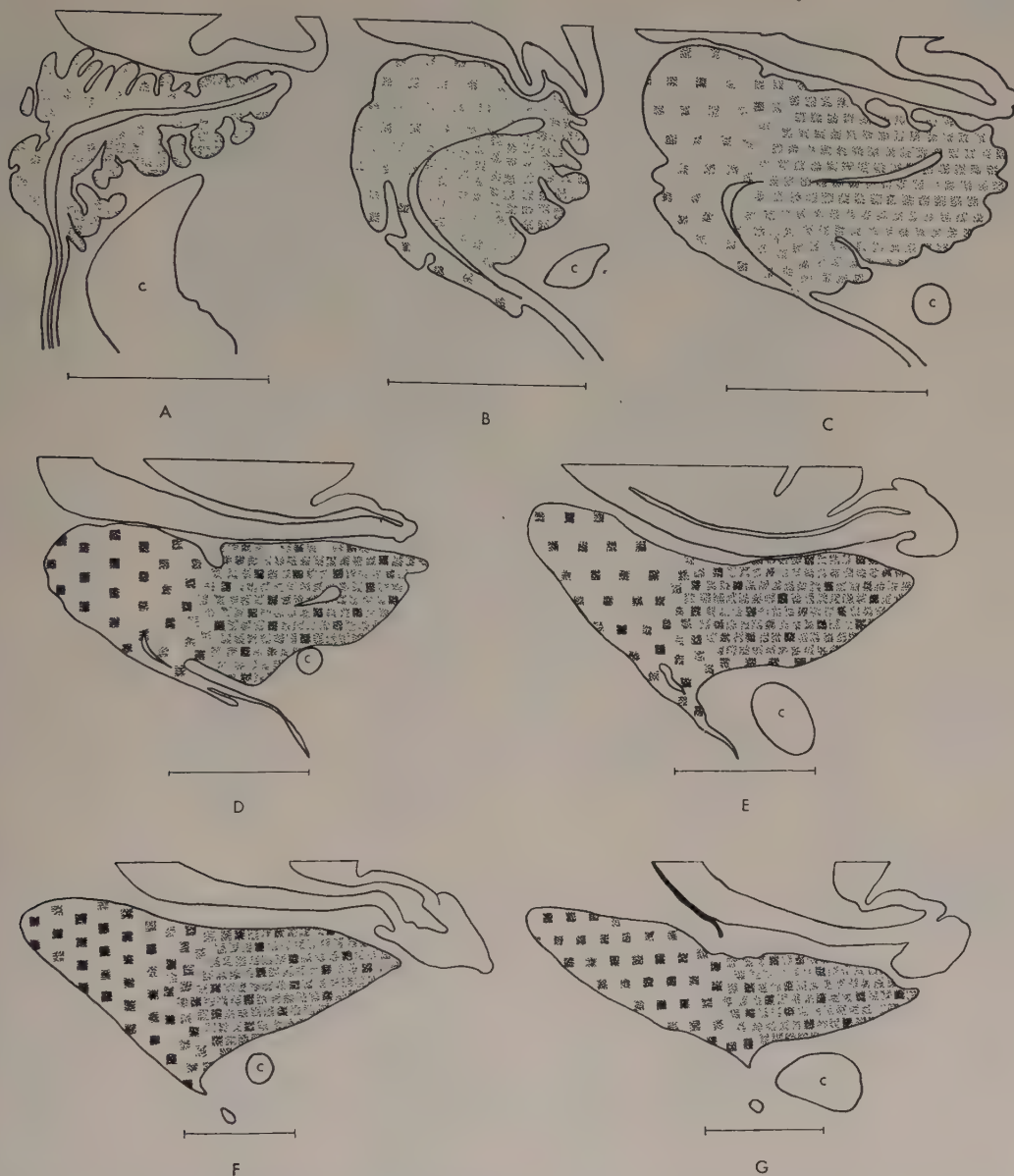


Fig. 64. *Gallus gallus*. Camera lucida drawings of median sections of the pituitary, showing diagrammatically the development of the histological bipartition. The frequency of the stippled squares and the density of their black dots are in rough proportion to the frequency and degree of granulation of the Ag-cells in the sections. Note the constant relationship between histological parts and the oral and aboral lobes and lumina. Compare also fig. 56 for a morphological interpretation. The glands drawn there are not the same as those drawn in this figure. The intercarotic anastomosis (c) has only an approximate relation to the border between the histological lobes.

Age of the embryos: A = 6 d, B = 8 d, C = 10 d, D = 12 d 4 hrs, E = 15 d, F = 19 d, G = 22 d (1.5 d after hatching).

Technique for all the sections: Bouin, paraffin 4 μ , Bodian. The line under each figure represents 0.5 mm.

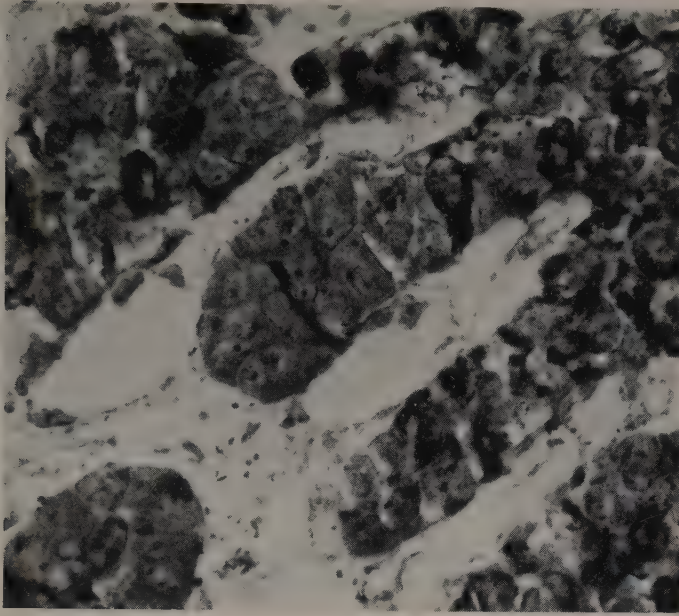


Fig. 66. *Gallus gallus*, embryo 15 d. Cell strings in the cephalic (oral) lobe of the pars distalis. The acidophils are grey (in the preparation red) and do not contain any silver-reducing granules. The Ag-cells are pure black. The string in the centre contains almost exclusively acidophils. — Bouin, paraffin 4 μ , Bodian + azan. Red filter to reduce the contrast of the dark red acidophils. 1200 $\times$.

10-day embryos. Later the acidophils become the most common type of cells there. The Ag-cells are much fewer and this agrees with the fact that the basophils remain relatively fewer throughout. I thus maintain that the origin of the chromophilic cells as it is shown in fig. 65, is well supported by facts. There are some doubtful points in the diagram, however, one is the origin of the bleach "neutral" cells which lack argyrophilic granules. Most probably they develop directly from indifferent embryonic cells and the acidophils should thus lack Ag-granules throughout their development. It is, however, theoretically possible that they first pass very rapidly through a granulated stage and then lose their argyrophilic granules as indicated by the query in the diagram. Anyhow, it is impossible that the granulated stage should last for a long time, for then the Ag-cells should be much more numerous in this part of the gland.

The caudal lobe presents a very uniform picture on the 11th day of incubation. The great majority of cells contain argyrophilic granules and their plasma assumes a pale "neutral" colour after azan staining. A relatively small proportion of cells with more densely packed argyrophilic granules show a basophilic tendency, but it is uncertain whether they can be referred to this category.

Further progress of differentiation, appearance of caudal lobe acidophils. In the

12- to 15-day embryos the acidophilic differentiation spreads throughout the cephalic lobe. These acidophilic cells in the 15-day embryo are very large and chromophilic, giving a predominant red colour to this part of the gland in the azan-preparations (fig. 66). The nuclei of the cells are rich in chromatin and look dark red in contrast to the nuclei of the basophils which are light and vesicular, usually with two distinct nucleoles. The basophils remain relatively few in the cephalic lobe and though some typical basophils have developed in the caudal lobe of the 12-day embryo they are very few in this part of the gland. Most basophils contain numerous argyrophilic granules, but in the 15-day embryos some of them do not, as can be observed sometimes also in the adult. The origin of these Ag-negative basophils is not clear. They may develop directly from embryonic cells but also from common basophils. Some of them are highly chromophilic and may be degenerating.

The first acidophils of the caudal lobe were observed in 15-day embryos and in the following days of incubation they increase rapidly in number. As mentioned above the caudal lobe has assumed a very uniform appearance already in the 9- and 10-day embryos, and in the 11- and 12-day embryos the cells are, with very few exceptions, filled with scattered, silver-reducing granules. Apart from the development of some basophils and a general increase in the density of the argyrophilic granulations this condition persists also in the 15-day embryos. Already in the 12-day embryo the cells tend to become long and columnar, in contrast to the cells of the cephalic lobe which remain more cubic or only slightly columnar. In the Bodian preparations of the 15-day embryos some caudal lobe cells have a changed appearance. Black argyrophilic granules are still present, but they occupy a peripheral position in the modified cells. The central parts of plasm are also impregnated, but here the gold is precipitated in a red colloidal state just as in the adult A₁-cells. If the gold is removed it can be shown by azan staining that the cells contain abundant acidophilic substance. These caudal lobe acidophils are few in the 15-day embryo, but very common in the 19-day specimen, and are the predominant type of cells in the caudal lobe of the newly-hatched chick. These cells are, however, difficult to examine in detail because of their slender, chromophilic cell-bodies, which shrink very much during the treatment with different solutions. They therefore look fairly compact, especially in the azan preparations, and it is difficult to see distinct granules in the acidophilic substance. The nuclei are dark and compact as those of the cephalic lobe acidophils.

It should be noted here, that the caudal lobe acidophils, from their first appearance on, are fundamentally different from the cephalic ones. The latter lack argyrophilic granules at an early stage of differentiation, and probably never contained any, whereas the former develop from typical Ag-cells. The black argyrophilic granules are preserved for some time even after the caudal lobe acidophils have turned chromophilic, but are later lost. These cells can, however, still be recognized in the Bodian preparations by the red impregnation of the acidophilic substance (cf. fig. 68).

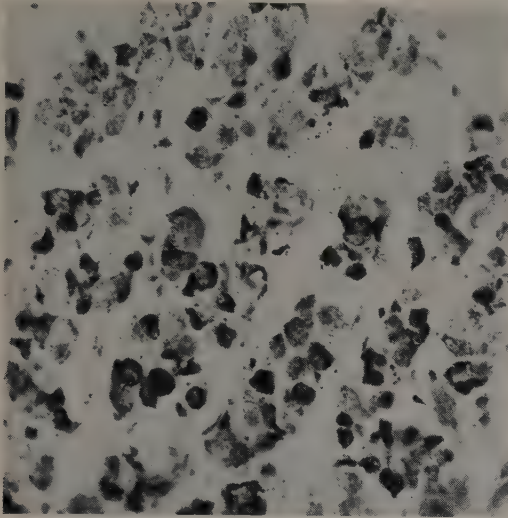


Fig. 67.

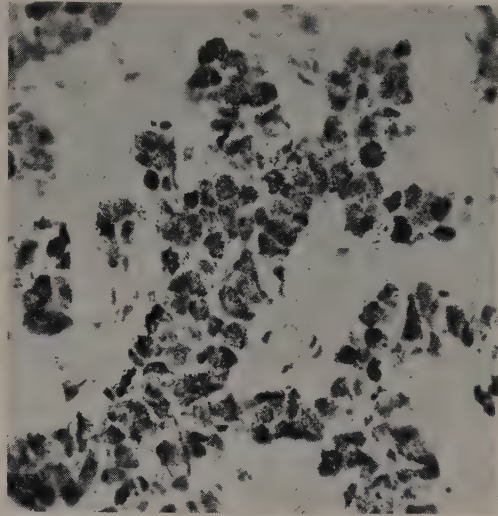


Fig. 68.

Fig. 67 and 68. *Gallus gallus*, 1.5 d after hatching (= fig. 64: G). Bodian impregnation to show the difference between the cephalic lobe (fig. 67) and the caudal lobe (fig. 68). The cells of the caudal lobe all contain Ag-granules and the picture is therefore more uniform. The cells of the cephalic lobe are mainly non-granular (A_2 -cells), and the Ag-cells here are frequently darker than in the caudal lobe. — Bouin, paraffin 4μ , Bodian, $560\times$.

The undifferentiated embryonic cells, just as the pale neutral cells, become rare after the 15-day stage. Some little stainable cells are present also in older embryos, but they are rare and their origin is not clear. The possibility that some chromophobic cells may develop from granular cells which have completed a secretory cycle must also be considered.

Hatching and post-embryonic development. In the newly-hatched chick the histological appearance of the pars distalis is largely the same as that of the adult. The cephalic lobe consists mainly of acidophilic cells which stain more deeply than the A_2 -cells of the adult, but agree with the latter in lacking argyrophilic properties. The basophils are not very numerous and do not look fully developed. They sometimes contain also a few reddish granules. It is difficult to classify any cells as chromophobes (cf. above), but similar difficulties exist also in the adult.

The caudal lobe is dominated by acidophils which have the same staining properties as the adult A_1 -cells. They are, however, not so distinctly granular as in the adult and are more slender with less cytoplasm. There are only few basophils in this part of the gland.

I have not studied the post-hatching development myself, but Rahn (1939) has described the changes which occur. The cephalic acidophils are said to be gradually replaced by cells with finer granules, the so called A_2 -cells. Whether this replace-

ment occurs due to a change of the embryonic acidophils or the latter are replaced by *other* cells is not definitely stated. It seems highly probable, however, that the A_2 -cells are merely the result of a transformation of the embryonic acidophils, because the embryonic acidophils agree with the A_2 -cells in lacking argyrophilic granules. Further, these types of cells predominate in the cephalic lobe of newly-hatched and adult specimens respectively. The embryonic acidophils are, consequently, the only kind of cells in the cephalic lobe which can give rise to the A_2 -cells without extraordinarily rapid multiplication.

The acidophilic cells of the caudal lobe are undoubtedly directly transformed into A_1 -cells. The fully differentiated basophils are said to appear simultaneously with the onset of sexual maturity (Rahn 1939) or on the 10th day after hatching (Payne 1942).

The pars tuberalis has received very little attention in my descriptions, because it remains fairly unaltered from the 6—8-day stage. Some of its cells contain numerous argyrophilic granules already in the 6-day embryo, and this picture, characterized by alternating granulated and non-granulated cells, is essentially the same in all older stages up to the adult. Rahn (1939) found basophils and acidophils in the 14-day embryo, and in the 18-day embryo the acidophils were common. He states, however, that both elements are less chromophilic than those of the *pars distalis*. These cells could be distinctly seen also in my preparations of embryos from the second half of the incubation period. The basophils are small, often spindle-shaped, and packed with argyrophilic granules. The acidophils agree with those present in the cephalic lobe. These chromophilic cells disappear later and are not present in the adult.

Larus ridibundus

No doubt the histogenesis of the gull embryos agrees closely with that described above for the fowl. I admit, however, that for several reasons the conclusions are not so well supported by the material of this species. The value of the Bodian impregnation for histogenetical studies was realized too late to be used for all stages of *Larus*, and the treatment of the specimens is less uniform throughout. Further, the age of the embryos was not perfectly ascertained, as they were collected in the field. Anyhow, the measurements allow a rough grouping of the embryos and gives some idea of the degree of development.

It has not been possible to ascertain the very first appearance of the Ag-cells in *Larus*, since the smallest embryos impregnated according to the Bodian technique measures 28 mm (CR). The Rathke's pouch begins to proliferate in the 14 mm embryo, but no distinct chromophilic cells appear in the tissue of the *pars distalis* before the 26 mm stage. In embryos measuring 18—20 mm there are some darker cells which stain in a neutral colour just as the Ag-cells of young chick embryos. Though it is impossible to identify these cells with certainty without Bodian impregnation, a comparison with the fowl material indicates that Ag-cells are present.

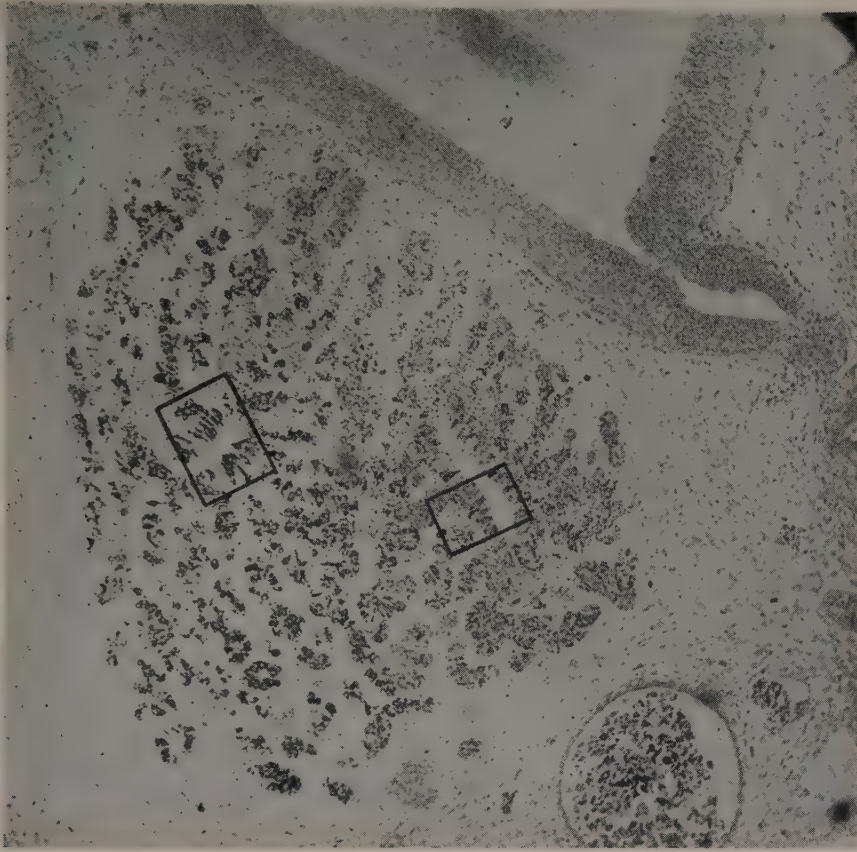


Fig. 69. *Larus ridibundus*, embryo CR 28, TL 45 mm. Paramedian section impregnated according to Bodian, showing the histological difference between the oral and aboral lobes (left and right resp.). The black ink squares including parts of the oral and aboral lumina are shown in high-power magnification in figures 70 and 71. — Bouin, paraffin 4 μ . Bodian. 115 $\times$.

Appearance of the chromophilic cells in embryos measuring 26—32 mm (CR). In most embryos of this size the basophils are distinct in the caudal lobe, and also acidophils can sometimes be identified. Bodian impregnation of two 28 mm embryos distinctly shows that the Ag-cells are present all over the gland and that the bipartition is extremely distinct (fig. 69—71). In addition to non-granular cells the cephalic lobe contains heavily granulated cells which appear almost compact black and few cells contain more scattered granules. Also some cells in the caudal lobe contain argyrophilic matter, but the granules are never very numerous and the cells therefore do not stand out so distinctly. The frequency of granular cells is slightly higher in the caudal lobe, but still there are many ungranulated cells in contrast to the chick. When the gold was removed in these two specimens azan staining showed that the dark Ag-cells of the cephalic lobe

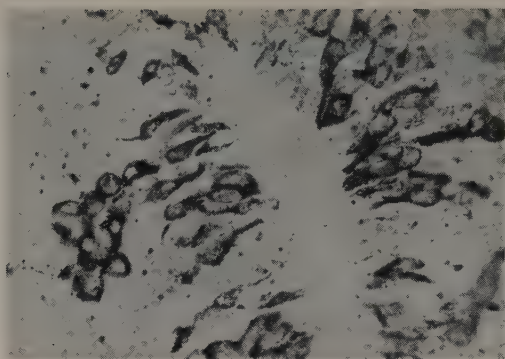


Fig. 70.

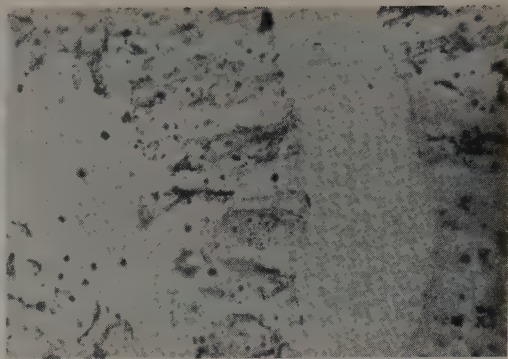


Fig. 71.

Fig. 70 and 71. *Larus ridibundus*. Ag-cells in the region of the oral (fig. 70) and aboral (fig. 71) lumina. Compare fig. 69. — Magnification, fig. 70, 540 $\times$, fig. 71, 650 $\times$.

contain numerous blue granules but also many red ones. They can therefore perhaps be classified as basophils but are not fully differentiated. In addition, some cells which lack Ag-granules show an increased affinity to stains. This gland is, in fact, very like a chick embryo after 10—11 days of incubation, just when the chromophilic cells begin to appear, but the border between the cephalic and caudal lobes is much sharper. In other preparations (CR 26, 27, 28, 29 mm) the basophils are quite distinct, and also typical acidophils can be seen in the cephalic lobe. The basophils are especially distinct in specimens fixed in Susa.

Also the pars tuberalis contains numerous Ag-cells in the 28 mm embryos, but it shows little affinity to stains in the azan preparations.

Further differentiation of the gland. Just as in *Gallus* the border line between the cephalic and caudal lobes is very little changed throughout the ontogenesis. This was obvious in the Bodian preparations (TL 64, TL 85). The basophils become more numerous and distinct but are restricted to the cephalic lobe. Not even in half-grown gulls measuring 28 mm total length are there any basophils in the caudal lobe. If Bouin, Zenker or Helly is used, the basophils in embryos up to about 90 mm total length become less clear. Though basophilic granules are distinct the cells also contain acidophilic matter which often makes interpretation difficult. The acidophils of the cephalic lobe increase in number and evidently change directly into the A_2 -cells whose typical shape and granulation could be seen for the first time in the young measuring 21 cm (TL). The cephalic lobe acidophils never contain any argyrophilic granules in this species either.

The argyrophilic granules are still present in the cells of the caudal lobe in embryos measuring 64 and 85 mm TL. The granules are not very densely packed, so that the contrast to the cephalic lobe is very sharp (fig. 72). The gull embryos seem to differ from the chick embryos in the high frequency of non-granular cells also in embryos of later stages. Thus, for instance about half of the cells of the

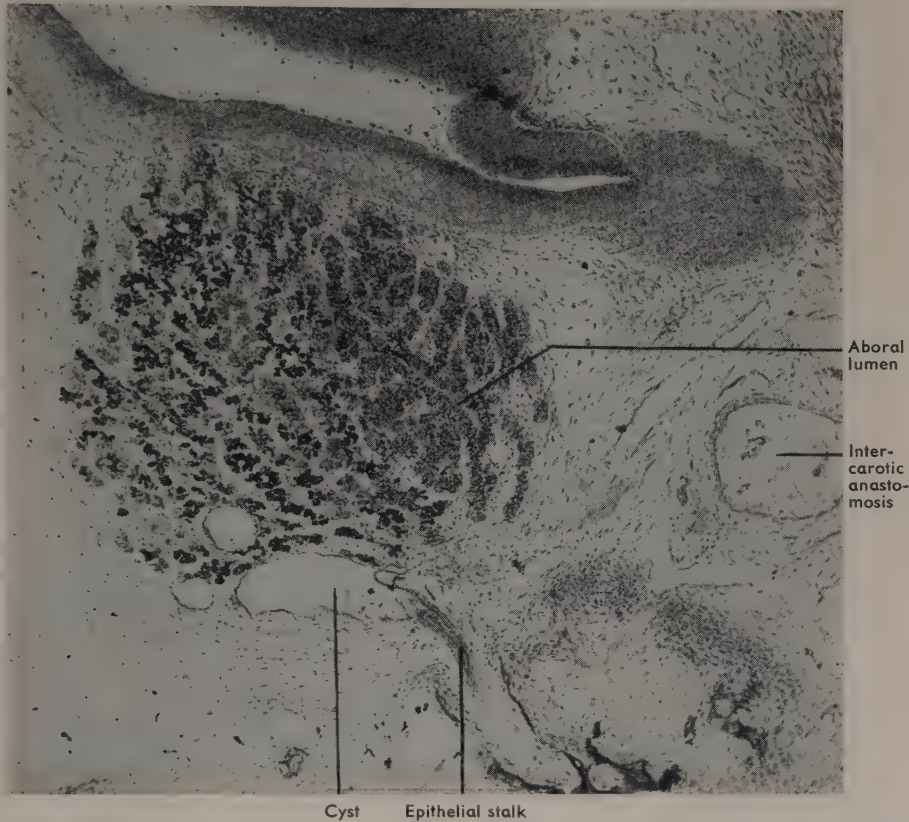


Fig. 72. *Larus ridibundus*, embryo TL 64 mm. Median section showing the histological bipartition of the gland as evidenced by the Ag-cells. The orientation is facilitated by a rest of the aboral lumen. — Bouin, paraffin 4 μ , Bodian. 100 $\times$.

caudal lobe are still non-granular in the 85 mm embryo (TL). Distinct acidophilic cells appear in the caudal lobe of embryos measuring about 80 mm TL and are quite distinct when the embryo has reached the hatching stage (about 100 mm TL). In the newly-hatched young they are numerous, and in a young measuring 13 cm TL they contain large flakes of acidophilic matter similar to those described by Voigt in the chick. In the three young measuring more than 20 cm TL the A_1 -cells are extremely chromophilic and packed with distinct granules. They are, in fact, the only type of granular cells in the caudal lobe in these specimens and, in addition, only a few non-granular cells of dubious origin are present. In the adult the caudal lobe also contains a few basophils, which certainly appeared there at the end of the growth period or later. It is remarkable that the basophils are absent from the caudal lobe throughout the ontogeny just as stated for ducks by Painter (1942).

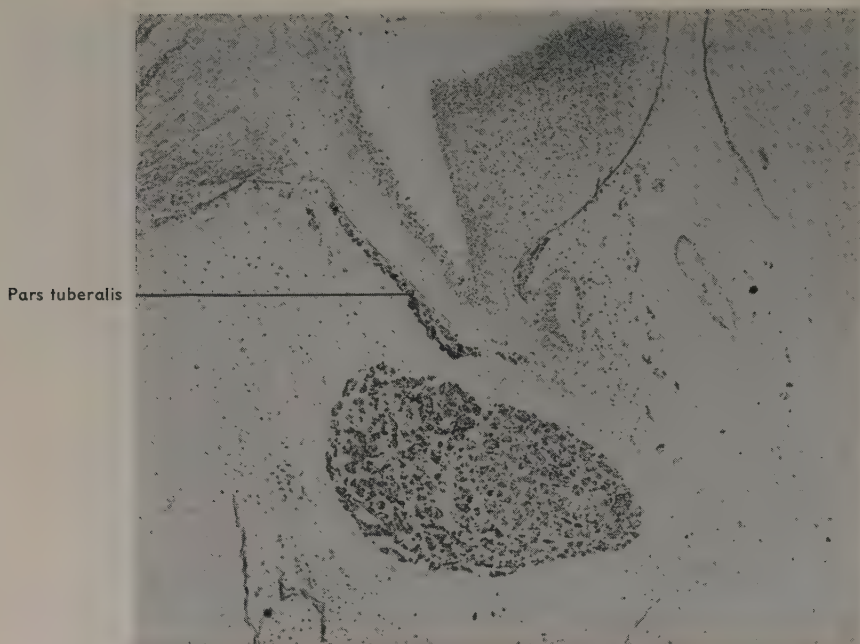


Fig. 73. *Riparia riparia*, newly hatched, CR 19, TL 30 mm. Paramedian section showing the bipartition. The cephalic lobe (left) contains darker Ag-cells than the caudal lobe (right). Also the pars tuberalis contains Ag-cells. — Bouin, paraffin 4 μ , Bodian, 100 $\times$.

Riparia riparia

Though a continuous series of embryos of this species is available and Bodian impregnation was used for a number of them it was difficult to state exactly the genesis of the chromophilic cells. It was possible, however, to fix their occurrence in relation to the stage of development of the embryo and to state their distribution within the anlage.

First occurrence of differentiated cells. Bodian preparations of embryos measuring 6 and 8 mm (CR) show no indication of cell differentiation. In an embryo measuring 9.5 mm (CR) the first Ag-cells were found. They are present only in the cephalic (oral) lobe, especially in its ventral parts near the attachment of the epithelial stalk. Also embryos measuring 10 and 11 mm contain numerous Ag-cells, but here some of them are present also in the caudal lobe, in the 11 mm embryo also in the basal parts of the lateral lobes. The azan preparations of embryos of this stage do not show any distinct cellular differentiation apart from an insignificant increase of the staining reaction of the Ag-cells.

Appearance of the chromophilic cells. The development of the chromophilia in *Riparia* seems to be continuous and slow. Though the chromophilia of the Ag-cells has increased in the 12 mm embryos they cannot be classified with certainty as

basophils before the 14 mm stage. Many of these cells also contain acidophilic matter in addition to the basophilic granules. At the same time also a few acidophilic cells can be seen in the cephalic lobe. They are pure red and lack basophilic matter throughout. These cells are all restricted to the cephalic lobe and they are not very distinct in the 14 mm embryo, but are clearly differentiated in the 15 mm embryos. In the 15 mm embryos there is still no chromophilia in the caudal lobe, and the Ag-cells in this part are very diffuse and not very numerous up to the 19 mm (CR) stage, when the embryo is hatched.

Further development and post-hatching changes. In the embryo at the hatching-stage (CR about 18—19 mm, TL about 27—30, fig. 73) the cephalic lobe contains a number of heavily granulated cells after Bodian impregnation. Some of the remaining cells contain a few argyrophilic granules, but most cells in the lobe do not have any granules at all. In azan preparations a number of more or less distinctly basophilic cells can be seen which seem to be identical with the Ag-cells. Also acidophils can be distinguished, but they are not as distinct as in the chicks at the corresponding stage. The caudal lobe of the full term embryos also contains a number of Ag-cells, but in contrast to the chick, there are also numerous cells without argyrophilic granules. Some cells, apparently corresponding to the Ag-cells with the densest granulation, have a distinct acidophilic character in the azan-preparations. In a young measuring 43 mm TL the numerous A₁-cells of the caudal lobe contain vacuoles with large acidophilic flakes and droplets, and such structures are also present in the cephalic lobe acidophils. The basophils are still restricted to the cephalic lobe. The changes from this stage to the adult are only slight — in the adult the acidophils of both types do not contain any red-stained flakes, and basophils occur also in the caudal lobe, though in smaller numbers.

Conclusions

Due to technical difficulties and to the nature of the problems the above descriptions are incomplete in many respects. It is very difficult to ascertain the changes occurring in the cells from the static and dead pictures seen in the fixed and stained sections. However, some interpretations are supported by such facts that they can be accepted as highly probable. I must emphasize, however, that complementary investigations are desirable.

1. The differentiation of the adenohypophysis starts much earlier than evidenced by the common azan preparations. The Bodian technique reveals differentiated cells in the chick embryo on the 6th day of incubation, whereas chromophilic cells are distinctly visible for the first time on the 11th day. Also in *Riparia* the Bodian technique reveals a differentiation before the true chromophilia has appeared.

2. The Bodian technique, which gives a picture of the gland characterized by the needed clarity, distinctly shows that the caudal and cephalic lobes develop independently and in very different ways. There are, in fact, two different glands composing the pars distalis of birds if we consider the histological structure and

its development. The difference between these two lobes appears at an early stage and remains constant throughout ontogenesis. Especially the closely spaced series of impregnated chick embryos illustrates this very clearly, but the results obtained from the two other species are in agreement herewith. This means that Voigt's idea of a continuously moving wave of acidophilic differentiation passing from the rostral to the caudal end of the gland must be regarded as inadequate. Certainly the differentiation begins at the rostral end as was also stated by Rahn (1939) and Painter (1942), but the bipartition of the gland is the primary feature to be considered.

3. The acidophils of the caudal and cephalic lobes are entirely different from their first appearance in the fowl. It is true that this conclusion could not be drawn from the material of *Larus* and *Riparia*, but, on the other hand, the scattered results obtained from these species do not speak against such an interpretation. In the cephalic lobe of the fowl embryo the acidophils differentiate from Ag-negative cells, whereas the caudal lobe acidophils develop from typical Ag-cells. Even if it is true that the acidophilic differentiation seems to spread over the border between the lobes it must be borne in mind that a different kind of cells is formed on the other side of the border.

4. Voigt (1939) described a kind of holocrine acidophilic cells in the pars distalis of young cocks. I have seen acidophilic cells with such large inclusions in the young of *Larus* and *Riparia*, but I have not been able to confirm the hypothesis of their holocrine function and have no reasons for regarding them as a separate type of cells.

5. The basophils are restricted to the cephalic lobe when they first appear, and particularly in *Larus* and *Riparia* even after hatching. This is also the case in *Anas* according to Painter (1942). The basophils contain distinct and numerous argyrophilic granules from their earliest stages of differentiation. Later some basophils appear which completely lack such granules, and similar Ag-negative basophils were also observed in the adult. The exact genesis of these exceptional basophils could not be cleared up. Whether their basophilic substance is identical with the stainable material of common basophils or not could not be decided.

6. We may thus conclude that the three types of granular cells in the avian pars distalis develop in different ways and partly also in different regions. Though the staining reaction of the cells may vary a little during the development it is impossible to regard the basophils and acidophils as different functional stages of the same cells. This view is also contradicted by the constant and regular distribution of the cells in the adult avian pituitary.

7. The origin of the chromophobes could not be followed. It does not seem probable, however, that they are the direct descendants of undifferentiated embryonic cells, for such cells are rarely if ever present in the full-term embryo of *Gallus*. The most probable origin of the chromophobes seems to be by degranulation of basophils and acidophils. Whether the chromophobes constitute a homogeneous category is open to some doubt. Some cells of this appearance contain

Ag-granules in the adult. Whether the chromophobes are able to form any kind of granular cells or whether each chromophobe is capable of forming one kind only is uncertain.

8. In *Gallus* and *Larus* there is a general strong acidophilia in the pars distalis of the full-term embryo. This was also noted for *Anas* by Painter and for *Columba* by Schooley.

9. The pars tuberalis begins to differentiate very early by development of Ag-cells just as the pars distalis. The present investigation also confirms Rahn's statement that chromophilic cells appear in the tuberalis of fowl embryos during the second half of the incubation period, and in fact both acidophils and basophils may be distinguished. An increased chromophilia of this part is visible also in *Larus* and *Riparia* at the corresponding stage, but no distinct differentiation into basophils and acidophils could be observed. The differentiation into Ag-cells and Ag-negative cells is preserved in the adult, but the chromophilia seems to disappear about the time of hatching in *Gallus*.

10. No specific differentiation of cells can be observed in that part of Rathke's pouch which corresponds to the intermedia of other animals. It has been suggested for whales and some other mammals that the failure to develop an intermedia depends on the presence of a thick septum of connective tissue which separates the adeno-hypophysis from the neural lobe (cf. Hanström and Wingstrand 1951). The idea is supported by experiments showing, that a close contact with the neural lobe is necessary for the development of the intermedia in amphibians (Blount 1945, Hegre 1946). The relatively thick membrane between the neural lobe and the adeno-hypophysis in birds could perhaps justify a similar explanation for the absence of the intermedia in these animals. However, there are often bridges of tissue between the aboral lobe and the neural lobe in the embryos which maintain the contact between the organs. The bridges are sometimes formed by the epithelial part, sometimes by nervous tissue, and were observed in embryos both of *Gallus* and *Larus*. Since a contact of this kind may persist also in the adult (cf. p. 71), without giving rise to a specific intermedia pattern it must be concluded that the absence of contact between the lobes is not the only factor which prevents the development of the intermedia. It must rather be concluded that birds lack the inherited dispositions for its differentiation.

Some Functional Aspects on the Histogenesis of the Pars distalis

There has been some discrepancy between observations on the ontogenetic occurrence of hormones in the avian pituitary on the one hand and the statements concerning cellular differentiation on the other. Chen, Oldham and Geiling (1940) showed that melanophore hormone is present already in the 5-day chick embryo whereas Rahn (1939) could not detect any histological changes in the pituitary until the 8th day of incubation, and the distinct chromophilic cells did not appear until the 11th day.

The results of Bodian impregnation described in the present chapter make it easier to compare the histological data. The argyrophilic granules distinctly show that differentiated cells are present in the chick embryo approximately at the time when the melanophore hormone is said to appear. It is true that I did not find any differentiation in the 5-day embryos, but distinct Ag-cells were present in an embryo incubated for 5 days 6 hours and in all older ones. Chen, Oldham and Geiling do not state whether their 5-day embryos had been incubated for 5 days exactly or not, and some differences depending on the breed of fowl used, on individual variation, and on the method of incubation can be expected. It is therefore not possible to regard a difference of 6 hours as significant, but the agreement of the results is exceptionally good.

The Ag-cells are the only type of differentiated cells recorded in embryos by the time of initiation of the melanophore-expanding activity and it is therefore reasonable to assume that they are responsible for the secretion of the hormone. Of course there is a theoretical possibility that additional types of differentiated cells can be demonstrated by refined technique but this possibility is indeed small.

As shown by De Lawder, Tarr and Geiling (1934) and by Kleinholz and Rahn (1940) the melanophore hormone of the adult chicken is produced mainly in the cephalic lobe. As the Ag-cells which are probably responsible for its production in early embryos are directly transformed into the basophilic cells, it is easy to suggest that the basophils are responsible for the production of the melanophore hormone in the adult. This is supported by the fact that the basophils of the cephalic lobe, together with a few chromophobic cells, are the only type of cells in this part of the gland which contain argyrophilic granules. It is admitted that we know very little about the significance of the argyrophilic granules, but the said facts could at least be cited in support of the theory, although they are no absolute proof. Kleinholz and Rahn (1940) are also inclined to attribute the melanophore-expanding activity to secretions from the basophils, but the reasons for their supposition are not clear. Of course it may be said that the melanophore hormone of some mammals is produced by the more or less basophilic intermedia, and this hormone should therefore be correlated with a basophilic reaction of the plasm. However, in the snakes the acidophilic intermedia has the same function.

Another function of the embryonic gland which has been discussed extensively is the production of the thyreotropic hormone. Rahn (1939) who found the first distinctly differentiated cells in 10—11 day embryos, did not believe that hormones from the pituitary initiate the activity of the thyroid, because this gland becomes active already on the 10th day according to the opinions at that time. Colloid was formed in the thyroid at the same time or even before the first active cells in the pituitary could be detected, and Rahn's deduction is therefore logic when considering the data available when the paper was written. Fugo (1940), however, showed that the formation of thyroid colloid is retarded in hypophysectomized embryos, and does not occur until the 14th day of incubation. Even then the amount of colloid remains very small and the droplets disappear again

before the end of the incubation period. In normal embryos the thyroid contains an increasing amount of colloid from the 10th day to the end of the incubation period. Further the thyroids of hypophysectomized embryos stop growing and practically do not exceed the size attained on the 14th day. Fugo concluded that the development of the thyroid of the fowl up to the 10-day stage is independent of hypophyseal function, but that the further differentiation and growth require hypophyseal hormones.

Fugo's experiments were beautifully corroborated by Martindale (1941). She transplanted thyroids from one embryo to the chorio-allantoic membrane of other embryos, and by using normal or hypophysectomized embryos as donors and hosts respectively she was able to conclude that the thyreotropic hormone is produced at least as early as the 11th day of incubation in the chick. She confirmed the earlier statements that the thyroid colloid appears on the 10th day in normal embryos and after 13.5—14 days of incubation in hypophysectomized ones.

In a summary of his own and other experiments Studitskii (1946) states that the first signs of secretion (accumulation and rarefaction of the colloid) occurs in the thyroids of the normal chick on the 8th day of incubation. He removed the pituitary rudiments from normal embryos of different age and grafted them on 7—10-day hosts in order to test the presence of thyreotropic hormone in the transplanted tissue. When the transplant came from an embryo of 10 days or more the reaction of the host thyroid was very distinct. Even when the donor was 8—9 days the host thyroid showed a distinct though less marked reaction. This would mean that the thyreotropic hormone is produced by the pituitary already on the 8th day, and the discrepancy with the previous statements concerning histogenesis is still greater. For more complete literature records I refer to Studitskii (1946), Martindale (1941) and Rahn (1939).

In my series of chicken embryos I have also examined the thyroids (fig. 75). The 10-day embryo has a distinctly active gland with extracellular colloid masses, though they are fairly small. The amount of colloid increases in the older specimens. Even the 9-day embryo shows distinct signs of thyroid activity. There are no extracellular colloid accumulations, but some cells contain distinct droplets, staining blue in azan, in their plasma. As these droplets must have arisen on the 9th day of incubation (the embryo had been incubated for exactly 9 days) my observations tend to confirm Studitskii's statements.

Comparing the development of the thyreotropic activity with the histogenesis of the pars distalis it will be seen that the initiation of the thyroid, at least in the breed of fowl used in the present investigation, occurs on the 9th day of incubation, and the thyreotropic hormone must, of course, be present before that time. No chromophilic cells are present on the 8th and 9th day, but a great number of Ag-cells, which begin to show increased stainability. This increased affinity to stains was also observed by Rahn (1939). The true chromophilic cells appear about two days after the thyroid has become active, on the 11th day. On the 9th day we

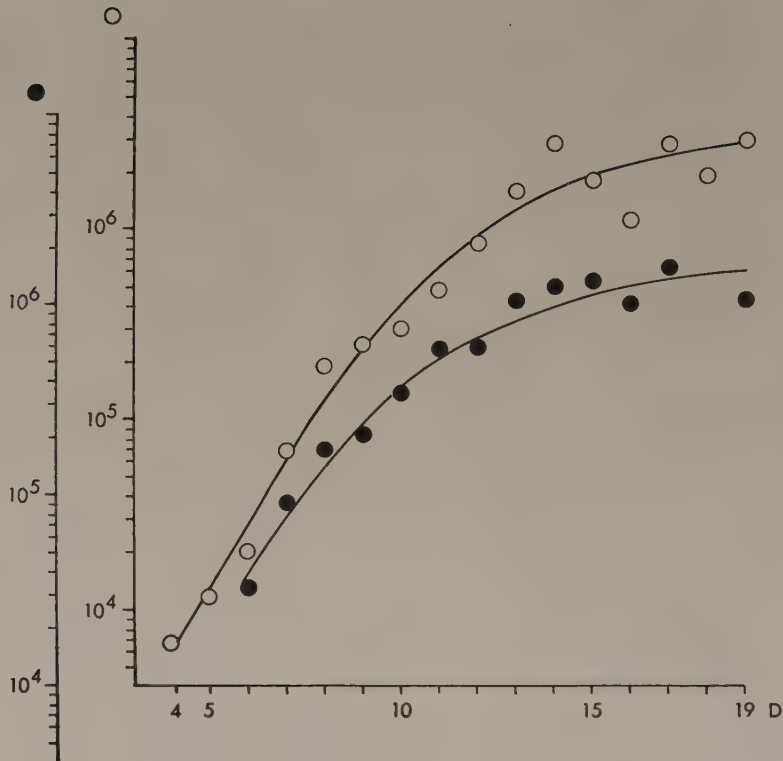


Fig. 74. Graph showing the growth of the adenohypophysis (black circles) and the thyroid (open circles) in embryos of *Gallus*. The age in days is plotted on the horizontal axis, and there is only one specimen from each day. The two vertical scales to the left are logarithmic but the figures there give the actual number of cells in the organs. It can be seen that there is no increase in the growth rate of the thyroid after the 9th day but rather a distinct decrease.

Note: The number of cells in the organs was calculated from the volume and the number of cells per cubic millimeter. The volume was determined by paper reconstruction of the section series according to the method of Hammar (1906, 1914, cf. also Floderus 1944, p. 89). The number of cells per mm³ was ascertained by cell counts in each specimen. Thereby the number of nuclei in 40 squares measuring 50 × 50 μ² was stated. The squares were distributed within the sections and section series so as to represent all parts of the organ proportionally to the volume. Then the number of cells per mm³ was calculated according to the following formula which is easily derived (cf. also Floderus 1944, p. 93):

$$N = \frac{n \cdot 10^7}{(a+d-k) \cdot 25}$$

where N is the number of cells per mm³, n is the mean number of nuclei in one square, a is the thickness of the sections, d is the mean diameter of the nuclei and k is a constant. The value of k was obtained from cell counts in 4 μ as well as in 8 μ sections, whereby

$$k = \frac{a_8(4+d) - a_4(8+d)}{a_8 - a_4}$$

if a_4 and a_8 are the mean number of nuclei per square in 4 μ and 8 μ sections respectively. The formula can easily be obtained from the one above if it is realized that the number of cells per mm³ must be the same either the counts are made in 8 μ or 4 μ sections. The value of k was about 1.0 μ for this material. It should be noted, however, that other values can be obtained from other material, other staining methods and by other investigators, for it is an expression of the minimum fragments of nuclei which are recognizable by the observer.

have, in addition to the Ag-cells, some Ag-negative cells with a slightly increased stainability, but they are few and in the 8-day embryo even rare.

The results obtained may, with some degree of probability, be combined in the following way: The thyreotropic hormone is produced by the Ag-cells from the 8th day on approximately, and this activity causes a slightly increased stainability of these cells. When the thyroid has been initiated and begins to produce considerable amounts of thyroxin this undoubtedly checks the output of thyreotropic hormone from the pituitary. This means that the thyreotropic hormone or, more probably, its precursors, are stored in the cells of the pituitary. The stainable substance which in the 10—11-day embryos is concentrated in the Ag-cells and makes them look as true basophils, may be such stores of thyreotropic hormone and its precursors. The interval between the first sign of thyroid activity and the appearance of the basophils — 2 days — may be the time required for development of the function of the thyroid to full efficiency.

The acidophilic cells have been left out of the discussion above, and in fact it is difficult to attribute a thyreotropic function to them. On the 8th day of incubation the Ag-negative cells with a slightly increased "neutral" stainability are still very few. Though they increase in number on the 9th and 10th day they seem too few and appear too late to explain the thyreotropic function. As described in the preceding chapter these cells give rise to the acidophils of the cephalic lobe.

In the above mainly the effect of the pituitary secretions on the *secretory function* of the thyroids has been dealt with. Fugo's (1940) experiments, however, also showed that a functional pituitary is necessary for the *normal growth* of the thyroids after the 14th day of incubation. It does not seem certain that this effect of the pituitary depends on the thyreotropic hormone. My investigation shows that the influence of these pituitary secretions which presumably appear in the blood some days before the 14th day of incubation, do not cause any increase or other change in the mitotic activity in the thyroid, but only keep it on the normal level. Fig. 74 shows the growth of the pituitary in chickens compared with that of the thyroid. As the values plotted on the vertical axis are the logarithms of the absolute cell number in the organs, the increase from one day to another shows the relative growth, that is, the number of cell divisions which have occurred. It can be seen that the curves for the thyroid and the pituitary closely agree, in spite of the fact that the thyroid is self-differentiated up to the 14th day approximately and later is influenced by the pituitary. The effect of the hypophyseal hormones is thus not an acceleration of the growth of the thyroids, they only keep the mitotic activity on the normal level. If the hypophyseal hormones are absent the mitotic activity will probably be approximately nil after the 14th day, according to Fugo's results.

I tried to compare the results from fowl with the observations made on *Larus* and *Riparia* (fig. 75). Though the age of the embryos is less adequately determined and the series of embryos is not so closely spaced here, it is possible to state some important points of agreement. It can be observed that the thyroid in *Larus*

becomes active long before the basophils can be identified in the hypophysis. Lack of material made it impossible to state this relation exactly in *Riparia*, but here it could be proved that the appearance of the Ag-cells comes very early, long before the thyroid is active and the chromophilic cells have appeared. The timing of the histogenesis in these two species thus seems to agree with the conditions in the fowl at some points at least.

The pituitary — thyroid relationships during foetal life have been investigated carefully in the pig. Rumph and Smith (1926) could state that the thyroid of pig embryos does not contain any detectable amounts of thyroxin at the 7-cm stage but it is definitely active in producing metamorphosis in tadpoles at the 9-cm stage. Rankin (1941) found iodine for the first time in 7—8 cm pig embryos and thyroxin was demonstrated in embryos measuring 8—9 cm. Rumph and Smith were, however, not able to demonstrate thyreotropic hormone in the pars distalis in embryos measuring 14—16 cm, while extracts from 26—28 cm embryos gave a slightly positive result. They tried to explain this disagreement by assuming that the thyreotropic hormone is not stored in the pituitary of the younger foetuses, but is immediately liberated into the blood. As they found the gland histologically undifferentiated before the 26—28 cm stage they were not inclined to accept this explanation, however, but suggested the theory that the hormone necessary for the initiation of the thyroid was supplied by the mother. Later Nelson (1933) found that the basophils appear in numbers in embryos measuring 7—10 cm, and this suggests that the relationships are the same as in the fowl; as the thyroxin appears in embryos of approximately this size it could produce the basophils by checking the secretion of thyreotropic hormone. It is then difficult to understand, however, that Rumph and Smith failed to demonstrate any thyreotropic activity of these pituitaries, but perhaps the tadpole test is insufficient for such small amounts of hormone in the pig. Nelson (1933) does not discuss the possibility of a relation between the appearance of basophils and the activity of the thyroid, probably because of the previous negative physiological statements. Instead he suggests a correlation between the basophils and the growth hormone, which is said to occur in the pituitary at the same stage (Smith and Dortzbach 1929). As mentioned by Nelson and also on p. 140 in the present work it cannot be generally postulated that the basophils have the same function in all animals and my comparison between fowl and pig here is therefore somewhat uncertain.

We know too little about the relations between the histology and the function of the fowl pituitary during the second half of the incubation period for a fruitful discussion. We know that the acidophils appear in the cephalic lobe about the 10—11th day, and that the A₁-cells begin to differentiate about the 15th day. On the other hand, Fugo's (1940) experiments indicate that hypophysectomy causes retarded body growth and abnormalities in the development of the gonads, perhaps also parathyroids, in the older embryos. It is customary to correlate growth hormone and acidophils, but safe conclusions could hardly be drawn before more facts are available.

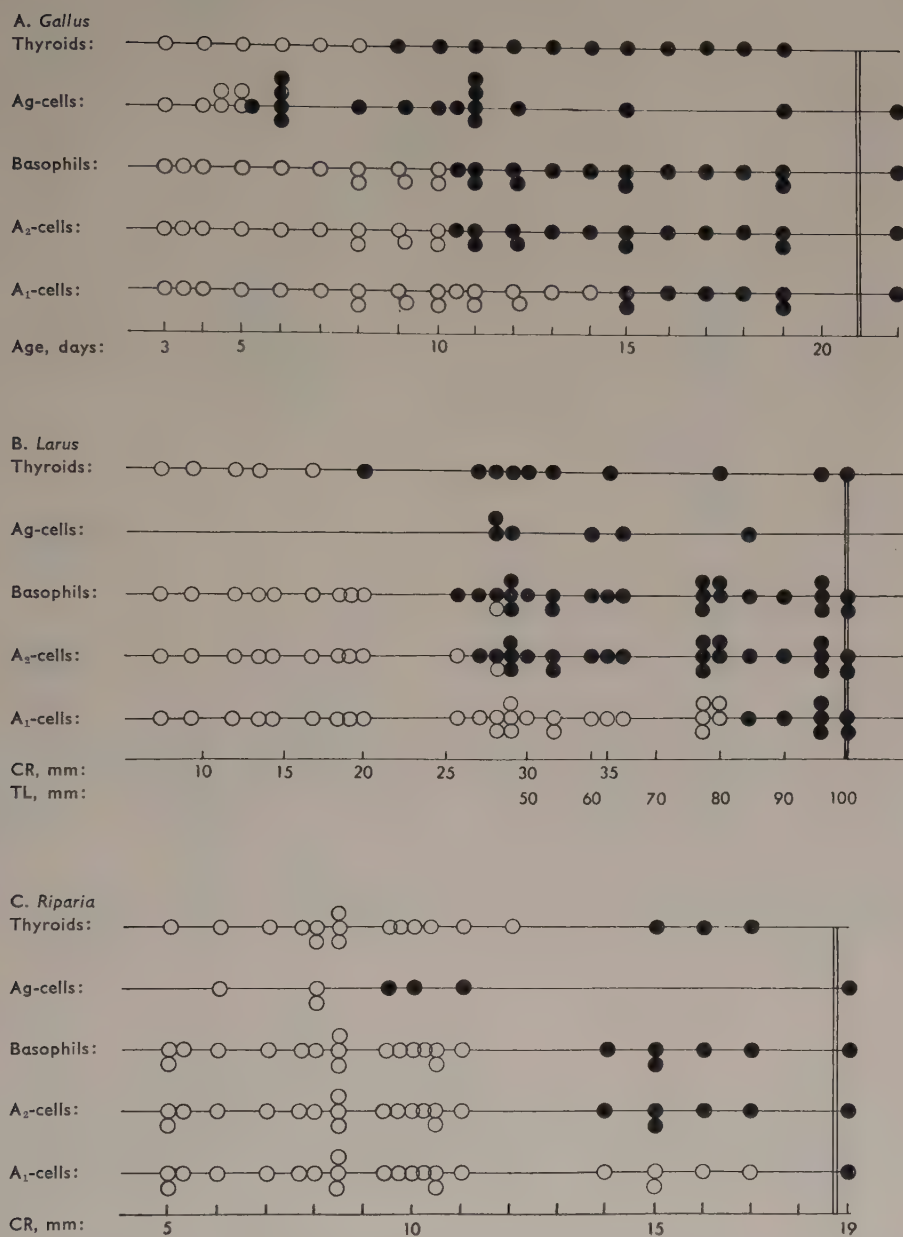


Fig. 75. Diagram showing the time of appearance of the cell types of the pars distalis in relation to the function of the embryonic thyroid. Each circle stands for one observation. Black circles denote the presence of colloid in the thyroid and the presence of the respective cells in the pars distalis. White circles denote negative finds. The double vertical line to the right marks the hatching-stage. The diagram A for *Gallus* is supported by the other two diagrams. Thus, in B (*Larus*) it can be distinctly observed that the thyroid becomes active before the appearance of the basophils, and in C (*Riparia*) the early occurrence of the Ag-cells is strikingly demonstrated.

CHAPTER V

The Morphological Plan of the Avian Adenohypophysis Compared with that of Reptiles and Mammals

Introduction

In my opinion a reliable morphological comparison of the adenohypophysis in different vertebrate classes is only possible on the basis of embryological studies. The series of diagrams on figures 43, 53 and 56 clearly shows that the different parts of the avian adenohypophysis can be traced from distinct portions of Rathke's pouch. These relations, found in the three investigated species, certainly have general validity within the group Aves, since the structure of the adult hypophysis in birds is very uniform (chapter III). It seems improbable that a different ontogenetic development could give an almost identical result in the different species.

Below is given a diagram showing the structural plan of the pituitary of a gull (fig. 76: B), which may be regarded as a typical representative of the group Aves. In addition, I have drawn the pituitary of an adult partridge, the embryonic development of which could be traced with a high degree of accuracy, because the lumen of Rathke's pouch is unusually well preserved in this specimen (fig. 76: C and D).

In general the morphological plan of the avian pituitary can be described as follows:

1. The caudal lobe is formed by the aboral lobe of Rathke's pouch, and it includes also the walls, which in early stages have the appearance of an intermedia.
2. The cephalic lobe is formed by the oral lobe of Rathke's pouch and its unpaired processes.
3. The pars tuberalis is derived from the lobuli laterales. The basis of the lobi is situated in the furrow between the caudal and cephalic lobes and here fuses with the pars distalis. These basal parts of the lateral lobes can sometimes be recognized in the adult as a pars tuberalis interna. The lobi laterales fuse more or less completely in the adult just dorsally of the pars distalis and form the portal zone. Their distal parts cover the surface of the eminentia mediana and the adjacent parts of the diencephalon, and a second fusion usually takes place behind the infundibular stem.

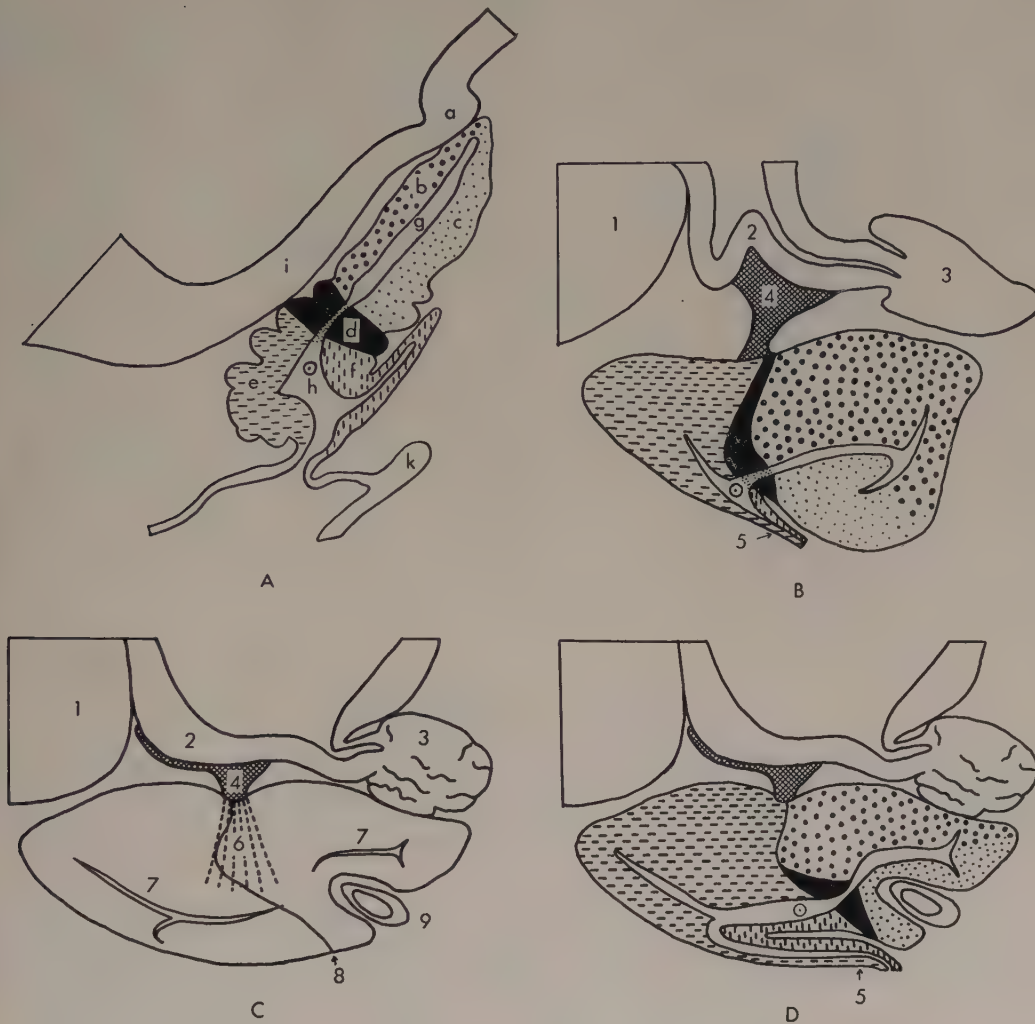


Fig. 76. Diagrams showing the development of the adenohypophysis in birds.

- A. Hypophyseal anlage in a 5-day chick embryo (*Gallus gallus*) showing the meaning of the different markings.
- B. Diagram showing the pituitary of a gull (*Larus ridibundus*, nearly full-grown young). The probable mode of development of the gland is indicated by the same markings as in A and by the addition of a lumen which is by far not so complete in the original. Confer also fig. 43.
- C. Pituitary of a partridge (*Perdix perdix*) showing the structures which indicate its mode of development.
- D. Author's interpretation of the same gland.

Legends to A: a = saccus infundibuli, b = rostral wall and c = caudal wall of the aboral lobe, d = walls of the constricted part, the narrowed lumen is stippled, e = rostral wall and f = caudal wall of the oral lobe, g = aboral and h = oral lumen, i = prospective median eminence, k = Seessel's pouch. The ring with the dot in the centre near h marks the point of origin of the lateral lobes as projected in the median plane.

Legends to B, C, and D: 1 = chiasma opticum, 2 = median eminence, 3 = neural lobe, 4 = pars tuberalis, 5 = epithelial stalk, 6 = pars tuberalis interna, projected in the median plane, 7 = residual lumen, 8 = histological limit of caudal lobe, 9 = anastomosis between the carotids.

4. The remnants of the epithelial stalk, if present, mark approximately one end of the border line between the caudal and cephalic lobes. The transformations which give this result will be understood if figures 56 and 64 are compared.

Woerdeman (1914) pointed out that the early differentiation of the pituitary anlage is very similar in different vertebrates. We are thus able to recognize the lobi laterales, the oral and aboral lobes and the constriction between them in embryos at a certain developmental stage, disregarding the taxonomical position of the species (the terms used by Woerdeman were somewhat different, cp. p. 76). If we are able to follow the development of the structures into the adult gland it is possible to compare the adenohipophysis of different animals with a high degree of certainty. This, of course, is true only if the said structures are really homologous, which, in my opinion, they are. The lobi laterales, which are paired, lateral diverticula, giving rise to the pars tuberalis in the groups treated here, are undoubtedly homologous. If the lobi are homologous this also supports the homologization of the constriction of the lumen just distally of their bases. And if the constriction is accepted as homologous throughout, this must also apply to the proximal and distal dilatations. Thus I think that the basis for the following comparison is fairly reliable.

Comparison with Lacertilia

The development of the pituitary in *Lacerta* is fairly well known thanks to Gaupp (1893), Baumgartner (1915), Woerdeman (1914) and De Beer (1926). As my own material of lacertilians mainly comprises adult specimens I have to base the comparison on the descriptions of these authors. When the pituitary anlage has grown out and formed the first processes it has the shape shown in fig. 77: B. It is roughly U-shaped with the epithelial stalk attaching to the bottom of the U. The caudal (right in the figures) leg of the U contacts the developing neural lobe and is thus the aboral lobe with the aboral lumen. A constriction of the lumen separates it from the oral lumen which has given rise to a large anterior diverticulum ("Vorraum"). The lobi laterales start from the oral lobe near the constriction just as in birds. The only apparent difference from the conditions in birds is the mighty development of the anterior process (the left leg of the U in the figures). In avian embryos this diverticulum is small or indicated only by a solid zone where the proliferation is intensive.

According to Baumgartner (1916) and De Beer (1926) the differentiation of this pituitary anlage takes place in the following way. The top of Rathke's pouch becomes flattened and forms the pars intermedia. The rest of the anlage except the lobi laterales, forms the pars distalis. According to Baumgartner the lobi laterales grow out and reach the brain, but are later reduced to two cell masses embedded in the surface of the brain and without contact with the main gland. I have seen these cell masses in some lacertilian species including *Lacerta agilis*, *L. vivipara*

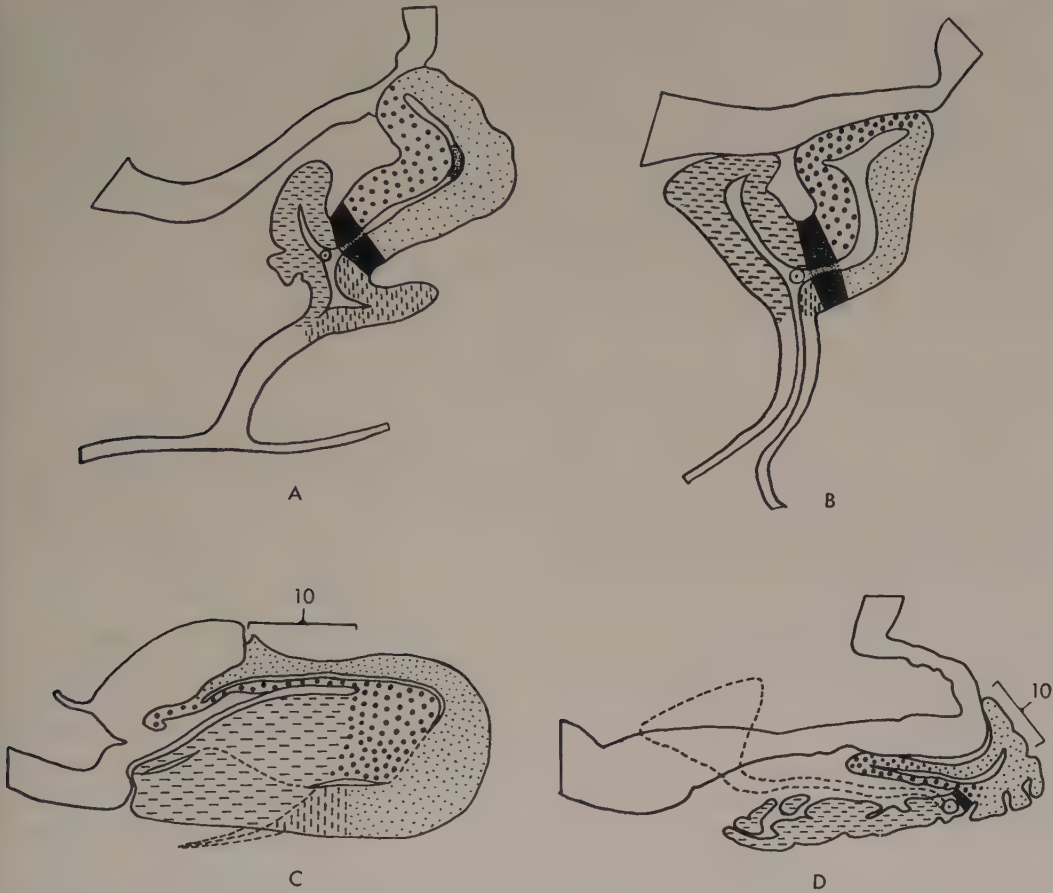


Fig. 77. Diagrams showing the development of the adenohypophysis in reptiles.

- A. *Vipera berus*, embryo TL 45 mm (tail included). The position of the ring marking the lateral lobes is very approximate (cf. text).
- B. *Lacerta muralis*, embryo 4.5 mm. After Woerdeman (1914, fig. 22). The position of the lateral lobes indicated according to the descriptions of Woerdeman.
- C. *Bitis arietans*, adult. Structures not present in the section are indicated by broken lines (the probable course of the lumen and the epithelial stalk). As the limits of the different areas are somewhat uncertain I have not marked out the black belt between oral and aboral lobes. 10 = pars intermedia.
- D. *Anguis fragilis*, embryo TL 80 mm (tail included). The course of the pars tuberalis which is not present in the median section, has been indicated by broken lines. The limits between the rostral and caudal sides of the oral and aboral lobes resp. could not be ascertained and the figure is, therefore, a little simplified. 10 = pars intermedia.
- For further explanation see fig. 76.

and *L. viridis*, but in some other species the tuberalis is completely reduced. Though the main features of the development have thus been described in the literature it is difficult to map up an adult pituitary of *Lacerta* in relation to the embryonic

structure of Rathke's pouch. The fixed points usually offered by the basis of pars tuberalis and epithelial stalk are lacking.

In some late embryos of *Anguis fragilis* in my collection the lobi laterales are still present in their full extension though they are entirely compact. The pituitaries can therefore be analysed in a more reliable way. As the gland has attained the definite shape at this stage, I choose one of these embryos to illustrate the features in adult lizards (fig. 77:D). As shown in the figure the aboral lobe of the anlage has formed the pars intermedia and the adjacent parts of the pars distalis. The rest of the pars distalis is formed by the oral lobe.

Comparison with Ophidia

The only reptile which I have examined in detail both embryologically and histologically is *Vipera berus* L., which is therefore chosen as a representative of the snakes. Though I have a closely spaced series of embryos of this species the development is difficult to follow, for the lobi laterales do not develop distinctly and the lumina behave in an abberant way. The median section of an early anlage is similar to that of lizards (fig. 77:A). The anterior process is distinct and gives the anlage the shape of a U. Also a caudal diverticulum has been formed from the oral lumen, and this seems to be very frequent in viper embryos. A comparison with *Lacerta* indicates that the lobi laterales should be searched for in the region marked by the ring. In fact, there is also a lateral extension of the lumen here, but it is not very distinct and cannot be identified with certainty as a pair of lobi laterales. There are two constrictions of the lumen, indicated by dots in the figure. The oral one has a position corresponding to the constriction of the *Lacerta* pituitary, and especially its position close to the anterior diverticulum speaks in favour of this interpretation. The aboral constriction is much narrower and cuts off the top of the pouch, which will later form the intermedia. This interpretation of the conditions in the viper is supported by Woerdeman's description of the pituitary in turtles. Here there are also two constrictions, the distal one cutting off the prospective intermedia, but the presence of distinct lateral lobes makes the interpretation easier.

During the further development of the viper embryos the top of Rathke's pouch proper forms the intermedia, and the aboral constricted part forms a slender stalk between it and the main gland. The proximal constriction becomes indistinct and the lateral lobes never grow out. The pars distalis must, of course, be formed by the entire anlage except the very top of Rathke's pouch which gives rise to the intermedia, but the exact share of the different parts of Rathke's pouch is difficult to make out. In fig. 77:C I have drawn the pituitary of the puffadder (*Bitis arietans*), which has a symmetrical hypophysis in contrast to most other snakes in which the gland is strongly asymmetrical. It can thus be presented in a sagittal section. This gland also contains considerable remains of the lumen which facilitate

the analysis. Unfortunately, the epithelial stalk was not retained in this specimen, but as it is present in another snake of the same species I have indicated its probable position by broken lines. Though the scheme given in fig. 77:C may be wrong in some details it certainly shows the main features of the snake pituitary correctly. The pars distalis is partly formed by the aboral lobe, but about half of it is formed by the oral one.

Comparison with Chelonia

Since my own material is still very poor I must here rely on the reports of Woerdeman (1914) and Baumgartner (1916). In embryos of *Chrysemys picta* the lobi laterales, the oral and aboral lobes, the constriction between them, and the rostral diverticulum appear regularly just as in *Lacerta* (Woerdeman). The distal portion of Rathke's pouch proper which will form the intermedia is cut off by a second constriction as in snakes. The further development in turtles does not seem to differ very much from that described for *Lacerta*, apart from the fact that the lobi laterales are preserved in the adult as a distinct pars tuberalis. It is thus evident that the top of the aboral lobe forms an intermedia and the rest of it takes part in the formation of the pars distalis. The oral lobe forms the remaining portion of the pars distalis, and seems to supply the material for a considerable part of it.

Comparison with Crocodilia

According to Baumgartner's (1916) and Reese's (1910) descriptions the adeno-hypophysis in alligators is probably similar to that in turtles. The lateral lobes are preserved in the adult as a pars tuberalis also in Crocodiles. A few remarks on Crocodiles in Woerdeman's paper (1914, p. 233—234) confirm the similarity with the Chelonians.

Comparison with Rhynchocephalia

The development of the pituitary of *Sphenodon* (*Hatteria*) is treated most thoroughly by Wyeth and Row (1923), and some remarks are also to be found in De Beer's monograph (1926). The former describe the early development in detail, and we find in their stage P the condition which has served as a starting-point for our earlier comparison. There is an anlage consisting of two lobes, one oral and one aboral, separated by a constriction. The lateral lobes originate from the oral lobe.

During the further development an intermedia is formed, and according to Wyeth and Row (p. 44) the aboral and oral lobes probably do not correspond to the intermedia and pars distalis resp. The authors were not able to find out the fate of these lobes because the anlage in older embryos is strongly folded and the constrict-

tion therefore becomes indistinct. This resembles the conditions in snakes, mentioned above.

A remark should be made here on the first appearance of the anlage in reptiles. Some authors (Gaupp, Wyeth and Row, Woerdeman) hold that the aboral lobe, the lobi laterales and the anterior process arise independently from the oral epithelium. Later this part of the epithelium is said to be folded off and form the oral lobe, into which open the lumina of the lateral lobes, the anterior diverticulum and the aboral lumen. In birds, however, the lateral lobes arise secondarily from the common, more or less tubular pouch. Baumgartner, however, after having studied the development in all orders of reptiles except the Rhynchocephalia, found a thickened area of ectoderm which is the common primordium of all three structures and which later is folded off as an oral lobe. He therefore could not regard the three structures as originally independent. As a matter of fact, there is, however, some difference between the reptiles on one hand and the birds on the other hand. The lateral lobes appear considerably later in the latter class, after the oral lobe is partly folded off. The difference thus depends on a delayed development of the lateral lobes and does not hinder the homologization of the structures in these groups.

A comparison between the structural plan of the adenohypophysis in birds and reptiles shows, that the caudal lobe of the adult avian pituitary corresponds to the intermedia and the adjacent part of the pars distalis in reptiles because the material in both cases is supplied by the aboral lobe. The cephalic lobe in the avian pituitary corresponds to the rostral part of the reptilian pars distalis, and this part is well developed in reptiles. The lobi laterales give rise to a pars tuberalis in birds, crocodiles, chelonians and Rhynchocephalia, but do not form a tuberalis in the snakes, in which they are indistinct already in small embryos, and they are partly or completely reduced also in lizards.

Comparison with Mammalia

The mammalian pituitary is highly different from that of birds and reptiles. Woerdeman (1914) tried to compare mammals and reptiles and found that the rostral parts of the mammalian gland are very much reduced. Only the aboral lobe is well developed and gives rise to the pars intermedia and the main part of the pars distalis.

The lower mammalian groups are, of course, of special interest for a comparison of this kind, and I shall therefore treat them so much in detail as allowed by the available material.

The pituitary of the *Monotremata* has been described by Hanström and Wingstrand (1951) and I refer to this paper for a complete account. The gross structure is also briefly described by Green (1951).

Fig. 78 shows a median section through the pituitary of *Ornithorhynchus anatinus* Shaw. The pars distalis is flattened, elongated in the median section, and a

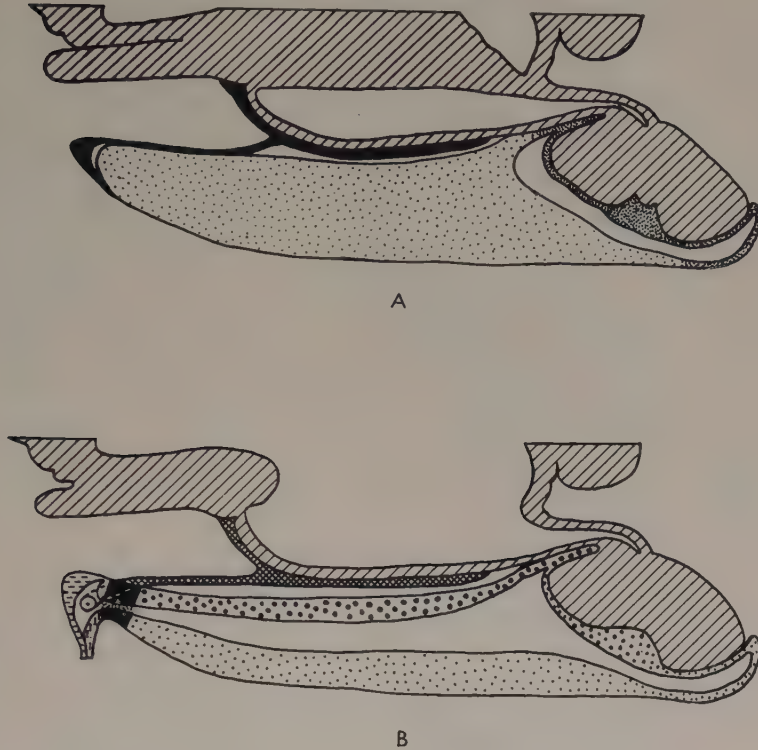


Fig. 78. The pituitary of *Ornithorhynchus anatinus*.

A. Median section of adult gland (coll. Hanström, Lund). The pars distalis is stippled, the pars intermedia dark stippled, the pars tuberalis and the chromophobic strings connected with it are black. The black areas have been made a little too broad relatively to demonstrate them clearly and they have been completed from 2—3 sections.

B. Diagram showing author's interpretation of the same gland. For explanations compare fig. 76.

distinct intermedia is present, separated from the pars distalis by a large hypophyseal cleft. The pars tuberalis shows a certain similarity to that of birds. It covers the median eminence as a thin layer and is connected with the pars distalis by a few very thin strings of cells (portal zone) which run rostro-ventrally to the main gland. When these strings reach the pars distalis they penetrate its capsule of connective tissue and can be followed further as a thin median string of chromophobic cells passing rostrally along the inner side of the capsule. At the very rostral point of the gland the chromophobic string is continuous with a body of chromophobic cells which is separated from the tissue of the pars distalis by a transversal cleft. It is my impression that this corresponds very closely to the conditions in birds and reptiles. The string of tuberalis cells from the eminentia to the pars distalis is undoubtedly the portal zone. Contrary to the conditions in birds it is bent rostrally and does not fuse with the pars distalis until near the

rostral end. This point from which the tuberalis issues must be situated within the oral lobe of Rathke's pouch, near its aboral end.

The cleft preserved in one of the specimens of *Ornithorhynchus* is perhaps the oral lumen of Rathke's pouch, and the chromophobic tissue at the rostral end must be derived from the oral lobe — it corresponds to the cephalic lobe in adult birds. Disregarding the development of an intermedia, the pituitary of *Ornithorhynchus* is thus a bird pituitary in which the cephalic lobe has become highly atrophied. The tuberalis is still attached to the border zone between aboral and oral lobes, but since the latter is small this zone has been dislocated towards the rostral end of the organ. Ziehen (1905) has described the embryonic development of the pituitary in *Tachyglossus* but does not give enough particulars to support the above interpretation. He states that Rathke's pouch of young embryos is divided into two lobes, one anterior and one posterior. Both lobes are said to be large and the pouch is said to be similar to the hypophyseal anlage in *Anguis*. This indicates that the oral lobe is larger in embryos of *Tachyglossus* than in the embryos of other mammals, but is later reduced.

The pituitary of *Tachyglossus* has also been described by Hanström and Wingstrand (1951). Also here the pars tuberalis has the bird-like appearance described for *Ornithorhynchus*. It is connected with the pars distalis by a portal zone consisting of strings which are independent of the infundibular stem. When these strings meet the pars distalis they deviate in rostral and rostro-lateral directions and do not fuse with the main gland at once. There is, however, no distinct chromophobic region nor any residual cleft at the rostral end. A considerable part of the anterior end of the pars distalis has a histological structure which is different from the caudal part, but the behaviour of the pars tuberalis shows that the two histological regions cannot correspond to the oral and aboral lobes in embryos. A detailed discussion will be found in the paper mentioned above.

Marsupialia. Fairly primitive conditions seem to be presented also by the pituitary of the *Marsupialia*, the development of which was excellently described by Parker (1917). The lumen of the anlage is here, at an early stage, subdivided by a constriction which separates an aboral lobe (distal lobe, Parker) from an oral (proximal) one (fig. 79). The aboral lobe proliferates and forms the intermedia and the pars distalis. The oral lobe, which carries the lobi laterales, remains small and the walls are thin. The epithelial stalk is, of course, connected with the oral lobe. Parker's figures and descriptions suggest that the oral and aboral lobe, the constriction between them and the lobi laterales are homologous with the equally named structures in birds and reptiles.

During the following development in *Trichosurus vulpecula*, according to Parker's descriptions, the juxta-neural wall of the aboral lobe forms the thin intermedia which is characteristic of the marsupials. The pars distalis is mainly formed by the rest of the aboral lobe. The oral lobe remains thin-walled and a rest of its lumen can be refound even in fairly large embryos. This is important for the interpretation of the adult gland, since at this stage the pituitary has approximately

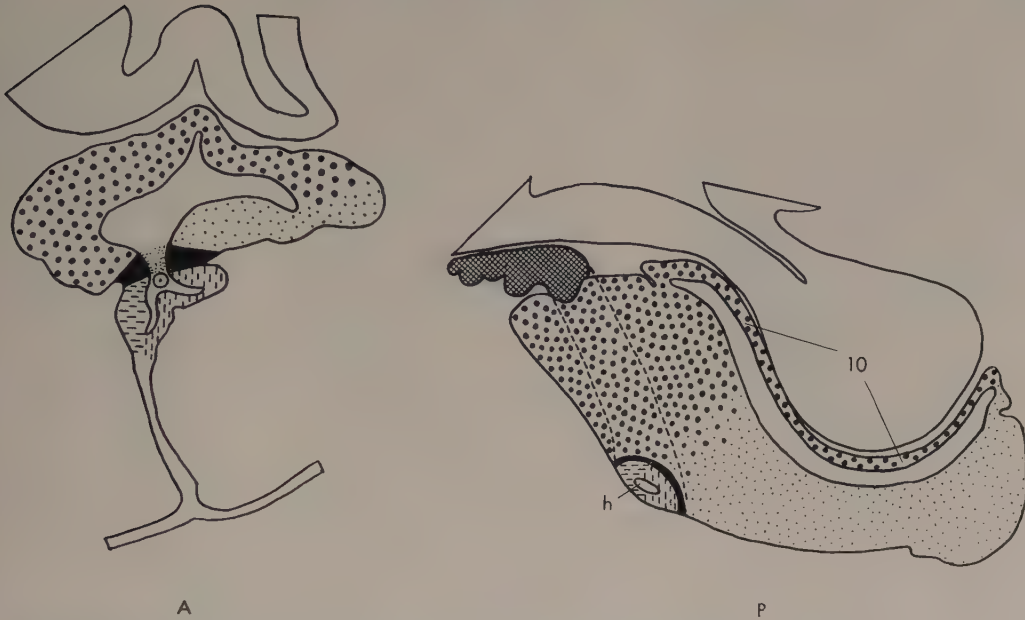


Fig. 79. Diagrams showing the development of the adenohypophysis in *Trichosurus vulpecula* (Marsupialia).

A. Embryo, stage VI, greatest length 11.5 mm. After Parker (1917, fig. 19). The ring marking the origin of the lateral lobes is added according to Parker's descriptions.

B. Pouch foetus, stage X, head length 12.5 mm. After Parker (1917, fig. 27). 10 = pars intermedia, h = oral lumen, which was also present in the preparations.

For further explanations see fig. 76.

attained its definite shape (fig. 79: B). The oral lobe is now embedded in the rostro-ventral wall of the pars distalis of the pituitary. Since it is positively stated in the text that the walls remain thin, and as Parker obviously paid attention to this circumstance, I cannot believe that the part of tissue formed by the oral lobe can be large. I have therefore only included a small zone around the vestigial lumen in the area marked as the oral lobe. The lobi laterales grow upwards along the sides of the pars distalis to the brain (eminentia mediana) where they fuse in the mid-line, and at later stages give rise to a complete pars tuberalis which forms a collar around the infundibular stem. The basal parts of the lobi laterales, which are indicated in the figure by broken lines, fuse with the sides of the pars distalis.

A comparison with the development in birds thus shows that the avian caudal lobe corresponds to the pars intermedia and the main part of the pars distalis in marsupials. The cephalic lobe of birds may be represented by a vestigial portion of tissue in the antero-ventral wall of the pars distalis of marsupials. The lobi laterales, of course, give rise to the pars tuberalis in both cases. It may be noted that the conditions for a paired pars tuberalis interna of the avian type are present since

the bases of the lobi laterales are separate and fuse with the sides of the pars distalis.

In the *Placentalia* the rostral parts of the pituitary anlage seem to be still more reduced than in the marsupials and monotremes. This can be clearly seen in young embryos, and there is little reason to believe that a proliferation of the proximal lobe should set in later in any species. The actual fate of the oral lobe is, however, often difficult to follow in higher mammals owing to the extensive proliferation of all parts in the later stages of development. I have therefore selected a few types of which the development is known, in order to illustrate the conditions in higher mammals.

The development of the pituitary in the rabbit (*Oryctolagus cuniculus*) clearly shows the degeneration of the proximal dilatation and its unpaired derivates. It is perfectly described in Atwell's famous paper of 1918.

Starting with the 13-day embryo we find a distinct pouch with the lobi laterales attached to the base (fig. 80: A). The border line between the aboral and oral dilatations should probably be searched for just above the bases of the lobi laterales as indicated in the figure. The lumen is constricted rostro-caudally about the middle of the pouch, but this constriction probably does not correspond to the lateral one in other animals. In the 14-day embryo (fig. 80: B) a compact epithelial stalk has been formed. The lumen in the region of the lateral lobes — the oral lumen — has disappeared, and there is a distinct lateral constriction just aborally of the lateral lobes, marking the boundary between the oral and aboral lobes. Atwell's interpretation of the dilatation between this constriction and the lateral lobes as "Mittelraum" shows that he was inclined to accept a morphological interpretation similar to that given here.

During the further development, the hypophyseal anlage is curved so the point of origin of the lobi laterales approaches the brain. The lobi grow out and reach the brain in the 16-day embryo. They fuse and form an unpaired cell mass between the main gland and the brain, and also the well-known epithelial cover on the brain surface. Atwell does not believe that the minute processus anterior ("Vorraum") contributes to these structures which are included under the term pars tuberalis. If we examine the 20- and 22-day embryos the residual cleft is left only in the region of the intermedia, but its probable course in the rostral part of the gland has been indicated in my figure (fig. 80: C). The part of the gland derived from the aboral dilatation can be identified with a high degree of certainty on the basis of Atwell's reconstructions, and it can be seen that this part occupies a very great portion of the gland. The remnants of the oral dilatation are difficult to delimit exactly but they have been indicated approximately in the figure by means of the fixed point offered by the epithelial stalk.

If we finally turn to the 30-day embryo which is said to correspond approximately to the adult we find that the epithelial stalk is connected with the extreme rostral end of the organ and that the remains of the oral dilatation must therefore be very small (fig. 80: D). Thus, in the rabbit, the main part of the gland including the

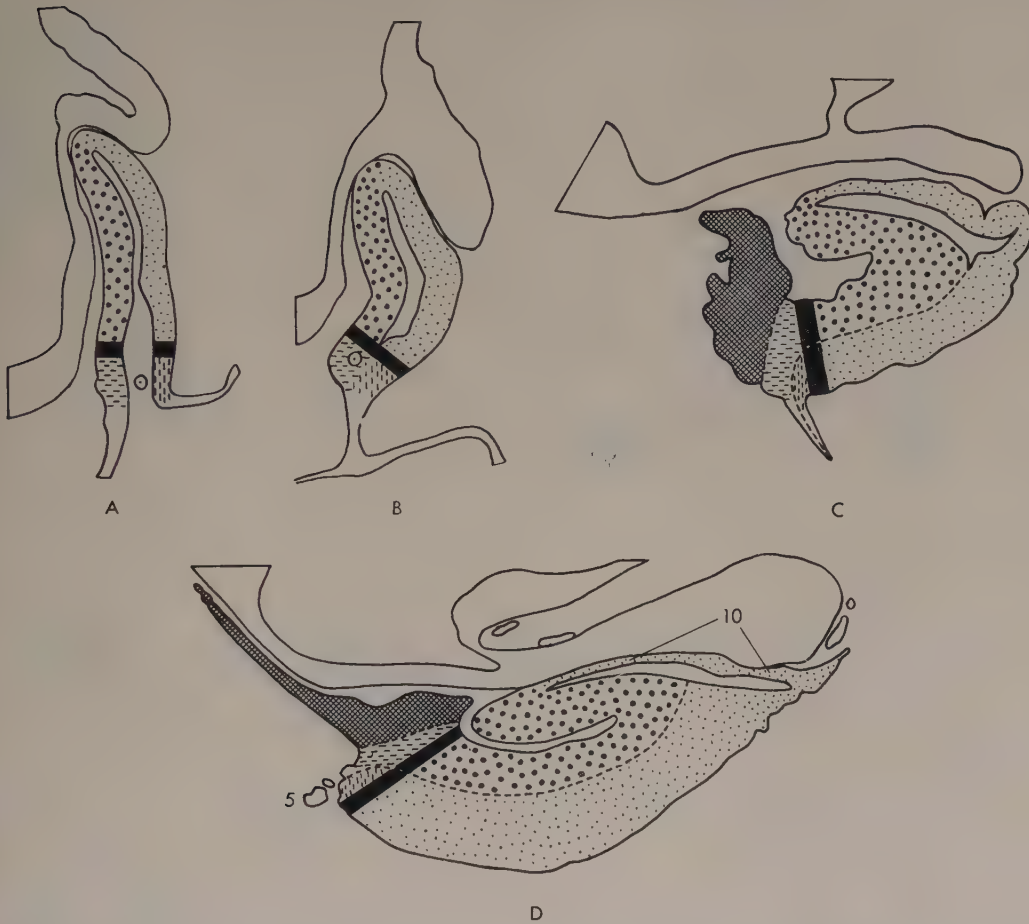


Fig. 80. Diagrams showing the development of the adenohypophysis in the rabbit (*Oryctolagus cuniculus*).

- A. Embryo, 13 days. After Atwell (1918, fig. 11).
- B. Embryo, 14 days. After Atwell (1918, fig. 13).
- C. Embryo, 20 days. After Atwell (1918, fig. 28).
- D. Embryo, 30 days. After Atwell (1918, fig. 35).

5 = remnants of epithelial stalk, 10 = pars intermedia. For further explanation compare fig. 76.

intermedia and the main part of the pars distalis are formed by the aboral lobe. The oral one must be left as a small portion of tissue near the base of the pars tuberalis at the very rostral end of the gland.

If we compare the development of the rabbit with that of the rat (*Mus norvegicus*) close agreement is found (Woerdeman 1914, Schwind 1928). The part of the anlage which carries the lateral lobes remains small and is dislocated to the rostral end owing to a curving of the gland similar to that in the rabbit (fig. 81: A and B).

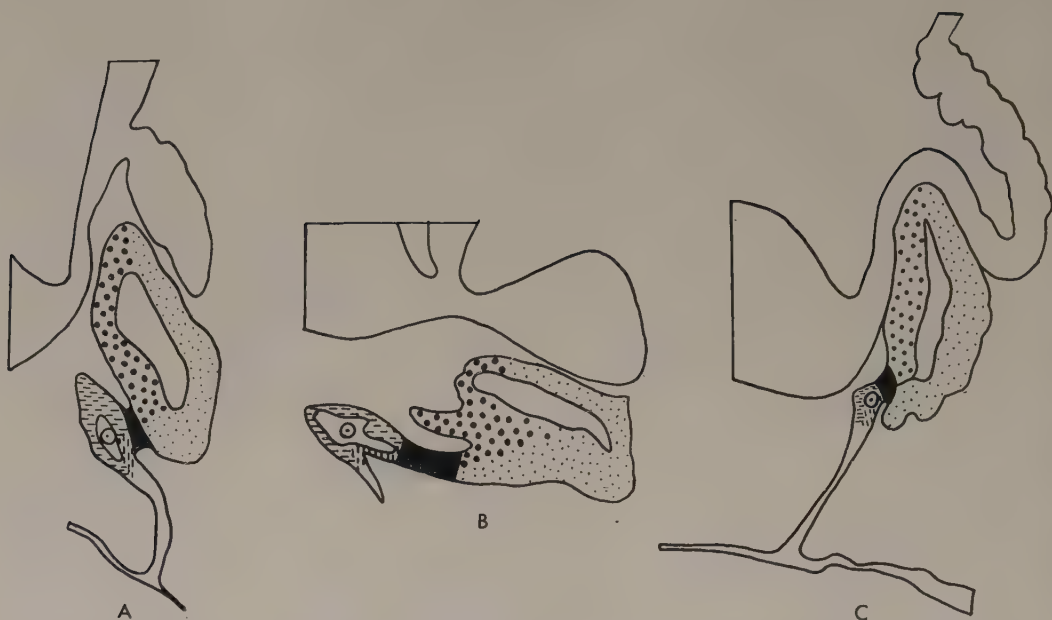


Fig. 81. Diagrams showing the early differentiation of the hypophyseal anlage in some rodents.
 A. Embryo of rat (*Mus norvegicus*), 11.5 mm. After Woerdeman (1914, fig. 5). Compare also Schwind (1928, fig. 8).
 B. Embryo of rat, 13.5 mm. After Woerdeman (1914, fig. 6). Compare also Schwind (1928, fig. 10).
 C. Embryo of guinea-pig (*Cavia cobaya*), CR 12 mm. Author's collection.
 For explanations see fig. 76.

According to Schwind the lumen of this part disappears at an early stage, but in Woerdeman's figures there is something like a lumen in it even at late stages. Also the development in *Sciurus* agrees fairly well with the descriptions for the rabbit (Woerdeman 1914), and some preparations of *Cavia cobaya* in my collection show that the oral dilatation remains small and becomes compact at an early stage in this species too (fig. 81: C). It may be noted that the constriction between the oral and aboral dilatations is more distinct in the rat and the guinea-pig than it is in the rabbit.

Kerr's (1946) description of the pituitary development in the laboratory mouse (*Mus musculus*) shows a general similarity with the scheme outlined above for the other rodents, but is difficult to analyse in detail. Perhaps the smallness of the animal has caused a simplification of structures.

Among the carnivores we may choose the cat (*Felis domestica*) as an example. The development of the pituitary in this species has been treated most thoroughly by Brahms (1932), and his descriptions are so clear that I have ventured to make some diagrams after his figures (fig. 82). He stated (p. 263) that the lumina of the hollow lateral lobes in the 11-mm embryo are continuous with that of the hypo-



Fig. 82. Diagrams showing the development of the adenohypophysis in the cat (*Felis domestica*).

A. Embryo 31.5 mm. After Brahms (1932, plate 2: 9).

B. Embryo 90 mm. After Brahms (1932, plate 4: 18).

10 = pars intermedia. For further explanations see fig. 76.

physeal stalk. "In other words, the lateral lobes and the hypophyseal stalk originate on a hollow ridge that is a slight evagination of the ventrocaudal wall of the hypophyseal sac." Probably this part of the lumen is to be regarded as a remnant of the oral lumen (cf. marsupials). As the figures show, the complex consisting of lateral lobe bases, remnants of epithelial stalk and the related lumen will remain in the antero-ventral wall of the gland and does not migrate to the very rostral tip as in the rodents. The lobi laterales grow up to the median eminence and form the pars tuberalis, and their bases remain as delicate strings of cells along the rostro-ventral surface (fig. 82: B). The remnants of the oral lobe should thus be looked for in the tissue near the ventral surface, a little rostrally to the middle of the gland. This conclusion is illustrated in fig. 82: B, and we may thus state a certain similarity with the conditions in marsupials. I admit, however, that the boundary between the aboral and oral lobes is uncertain in the figures, for nothing is said about constrictions etc.

I have myself examined a section series of a fox pituitary (*Vulpes vulpes*) in professor Hanström's collection in this institute and I found that the strings of the true pars tuberalis (not to be confused with the rostral protuberances of the intermedia!) continue as chromophobic strings along the rostro-ventral surface of the pars distalis, just as in the cat embryo on fig. 82: B. This indicates, in fact, that the pituitary of the fox is formed in the same way as in the cat.

Kingsbury and Roemer (1940) studied the embryology of the pituitary in the dog (*Canis familiaris*), but did not follow the formation of the pars tuberalis in detail. However, the fact that the epithelial stalk remains in contact with the ventral wall and is not dislocated to the very rostral end indicates a similar development as in the cat (cf. also Obussier 1944 concerning the epithelial stalk in the adult dog).

I hesitate to try a detailed comparison with the development in the other mammals in which it has been described because the uncertainty will be too great — either because of complication of the embryonic structures by proliferation, or because insufficient information is given. It seems, however, that the development of the pituitary in the pig (*Sus domestica*), the armadillo (*Dasypus novemcinctus*), the sheep (*Ovis aries*), in bats (*Myotis murinus*), and in man could be referred to the general type with some modifications. I refer the reader to the original descriptions (Oldham, 1941, for the armadillo, Hochstetter, 1924, for the bats, Haller and Mori, 1925, and Nelson, 1933, for the pig, Lubberhuizen, 1931, for the sheep, Hochstetter, 1924, and Atwell, 1926, for man).

The said examples tend to show, however, that the conditions in monodelph mammals are similar to those in marsupials and monotremes though the reduction of the oral lobe is still more advanced. The rodents seem here to offer the best material, since the constriction between the aboral and oral lobes is distinct in some species. The result of the development is that the aboral lobe gives rise to the intermedia (if present, cf. Hanström 1944, Hanström and Wingstrand 1951) and the main part of the pars distalis. The lobi laterales form a pars tuberalis and the oral lobe remains in a vestigial state either near the brain at the base of the tuberalis (rat, rabbit) or under the rostro-ventral surface of the gland (cat). In the latter case the bases of the lobi laterales must run along the surface of the pars distalis to the brain — a feature found also in *Trichosurus* and birds. It is not necessary that they should be paired, as in the said marsupial, however, but the two lobuli may fuse either with each other or with the processus anterior of the oral lobe (? sheep, Lubberhuizen, 1931).

A morphological comparison between mammals and birds thus gives the result that the avian caudal lobe corresponds to the entire epithelial gland of mammals, with the exception of course of the pars tuberalis which has its equivalent in birds, and a very restricted portion which may correspond to the cephalic lobe. The latter which is derived from the oral lobe is represented by some vestigial part of tissue near the rostral end of the mammalian gland or, in other cases, under its rostro-ventral surface.

Discussion

I have used considerable space for the ontogenetic comparison between birds, reptiles and mammals in this chapter, because the morphological relationships are so important for histological and functional aspects of the gland. And in spite of the fact that these relationships were discussed already by Woerdeman (1914) I found it necessary to make a detailed account, supported by new findings, because

recent investigators do not seem to have realized their importance. Moreover, Woerdeman did not carry through the comparison to the adult stage as I have tried to do here. Even before 1914 the structures used for the comparison had been discovered in many animals, and the homology of the lateral lobes had then been realized, but Woerdeman was the first to try a general interpretation (cf. his literature review). The ideas have later been criticized and as regards some details this criticism has certainly been justified (Atwell 1918, p. 331 and 1926, p. 172). The main conclusions concerning amniote vertebrates seem to be uncontradicted, and are in general the same as those arrived at above. However, I have avoided Woerdeman's subdivision of the oral lumen into "dorsaler Mittelraum", "ventraler Mittelraum" and "Vorraum" for the following reasons. The structures in question are difficult to distinguish or are inconstant in the groups treated. Further, it is almost impossible to follow these structures into the adult stage, and finally, the oral lobe of birds, which are the starting-point for the present comparison, gives rise to a morphological unity in the adult: the cephalic lobe.

In later papers Woerdeman's view has been defended mainly by Atwell, in his papers on the pituitary development of the rabbit (1918), of fowl (1918, together with Sitler, and 1939), and of man (1926). As regards the fowl he observed that the pars distalis is partially divided by a groove which lodges the internal carotids. He finds it possible that the caudal lobe might represent the "Rathke's pouch proper" of Woerdeman and the rostral lobe "would be a composite of Mittelraum, Vorraum and Ventralsäckchen". He is of the opinion that further research is necessary to decide the matter. Lups (1929) introduced Woerdeman's terminology for the pituitary anlagen of *Anas* and *Gallus*, and Rahn (1939) stated for the fowl that the portions in the adult, derived from the oral and aboral part of Rathke's pouch respectively, are histologically different. A similar statement was made by Painter (1942) for the duck. Neither of these authors did, however, try to compare the adult gland with that in other vertebrate classes with the ontogenetical results as a basis.

In a similar way the validity of Rathke's terminology was discussed for mammals by some authors (e. g. Atwell 1918, 1926, Schwind 1928, Lubberhuizen 1931). According to Lubberhuizen the anterior process proliferates considerably in the sheep. The conditions in this species are, however, very difficult to interpret and it seems questionable whether all Lubberhuizen's statements could be perfect. Especially the part of the lumen called "Vorraum" in the later stages (figs. 48, 51, 53) corresponds very well to the vestigial part of the aboral lumen in other mammals (compare my figures 79—82). The "Vorraum" in the young sheep embryos seems, however, to deserve this name.

In this connection it may also be mentioned that Parker's (1917) comparison of the oral ("proximal") lobe in marsupials with the lateral lobes in reptiles is slightly inadequate. As pointed out above this unpaired oral lobe, which gives rise to the lateral lobes, should be compared with the unpaired part from which the lateral lobes grow out in the reptiles; and this is the "Mittelraum" of Woerdeman.

CHAPTER VI

The Histology of the Avian Adenohypophysis Compared with that of Reptiles and Mammals

Introduction

The morphological comparison in the preceding chapter made it possible to establish a number of homologies for the different parts of the adenohypophysis in amniotes. In this chapter the morphologically distinct parts of the gland will be correlated with the distribution of different cell types, and the correlation, if any, between morphological partition and histology will be examined.

The theoretical aspects of such a comparison will first be considered. Firstly, are the elements used for comparison, i.e. the cells with the well-known staining reactions, really identical throughout in some fundamental respects? If the identical feature of the basophils, acidophils etc. is restricted to their staining reactions the comparison will be of little value. If, on the other hand, the staining reaction is a reliable indication of the function of the cells it will be possible to state whether homologous parts of the adenohypophysis have the same function throughout or not. If they have, it will be possible to use histological evidences for homologization as has sometimes been attempted. Most histologists are of the opinion that if two parts of tissue in the same pituitary differ in the appearance of their cell types, this usually indicates some difference in function. But, inversely, if we compare different animals and find cells with similar staining reactions in some or all of them, can these cells be assumed to be functionally equivalent? For mammals in which the cellular make up of the gland is very uniform throughout, this question could probably be answered in the affirmative, as long as we keep within this class. The same can be said about birds, but if we compare different classes of amniotes or if we investigate the reptiles the problem is far from clear.

Witschi (1937) made it probable that even the hormones themselves, especially the gonadotropic ones, have a slightly different structure in different animal groups. This is shown by the fact that heterozoic applications of hormones often fail to give good positive results. It is of course possible that also the cells giving rise to a certain hormone can be of a different appearance in animals not closely related. It is not justifiable to hold that generally the staining reactions depend on such

parts of the proteins which are essential for the hormone production. Maybe a certain protein serves the same purpose in many animals because its active groups are identical, but that other parts of the same molecule show specific differences which cause different staining properties of the cells.

As to the basophilic cells we have some support for the view that they serve the same purpose throughout. V. Soós (1935) pointed out that the basophilic properties probably depend on the presence of muco-proteins in the cells. He examined mammals and birds, but this conclusion can be extended also to the reptiles since the staining reactions of the basophils are very similar in this group. Moreover, it has recently been shown that the gonadotropic hormones are muco-proteins (Evans, Fraenkel-Conrat, Simpson, and Lee 1939), and Pearse (1948, 1950) could demonstrate by cytochemical methods that muco-proteins are present only in the basophilic and some chromophobic cells in man, sheep, and goat. These investigations make it probable that also a less specific reagent for muco-proteins as e.g. phosphotungstic acid — aniline blue used in the azan procedure preferably reacts with cells containing gonadotropic hormone and its precursors.

Another observation supporting the view that the basophilic cells are functionally equivalent is their similar reactions to thyroidectomy and castration in many mammals, birds and reptiles (cf. e.g. Romeis 1940 pp. 517—544, Sieler 1936, Satwornitzkaja and Simnitzky 1932, Schooley 1938, Payne 1940). Although the basophils of different amniotes may reasonably be regarded as equivalent, the matter is certainly complicated. Romeis (1940) clearly demonstrated two different kinds of basophils in the mammalian pars distalis, and V. Soos (1935) pointed out that the staining reactions of the basophils differ from one animal species to another. Moreover, the reactions of the basophils after thyroidectomy and castration are not perfectly the same in all vertebrates (Romeis 1940, Payne 1940). Some authors are not even convinced that the reacting cells are basophils.

The acidophils are far more difficult to interpret. In most thoroughly investigated amniotes there are at least two different kinds of acidophilic cells, but their interrelationship and function are inaccessible to discussion at present (Romeis 1940, Dawson 1946, Sieler 1936, Poris and Charipper 1938, Miller 1948, Hanström 1946). Any certain relationship between the chromophobes of different animals is also difficult to find except that they seem to be little active. The cells included in this category are probably of very different significance in different animals.

It has also been suggested that the basophils and acidophils are functional or developmental stages of the same cells (theorie unitariste, cf. review in Romeis, 1940, p. 124 ff.). This is impossible in birds in which the different cell types show a very constant and regular restriction to certain parts of the gland, and the same is true for many reptiles as will be shown below. As also investigators of mammalian pituitaries have abandoned this idea there is no reason to discuss it more in detail.

The assumption that at least some basophils are functionally equivalent in dif-

ferent vertebrate classes may justify an attempt at a general histological comparison in the Amniota, but of course the numerous uncertain points as regards the cell types make great caution necessary.

Comparison with Lacertilia

The histology of the lacertilian adenohypophysis is so variable that it is impossible to give a complete survey of it here. I therefore select a few types to illustrate some variations. A more complete account will follow in a later paper. We may begin with *Anguis fragilis*, which was examined morphologically in the preceding chapter (fig. 83: A). The pars tuberalis is vestigial in the adult and its cells stain faintly violet. The pars intermedia is decidedly basophilic, staining in a pure blue colour. The adjoining zone of the pars distalis is made up mainly of intensively staining acidophilic cells mixed up with a few basophils and very few chromophobes. The entire rostral part of the gland is composed mainly of strongly basophilic cells, but this region also contains an acidophilic cell type different from that in the caudal part, and a fairly large proportion of small chromophobes situated centrally in the strings. It may be mentioned that the basophilic cells of the rostral part of the gland are selectively stained by resorcin-fuchsine (R § 1561). The two types of acidophils mentioned differ in the appearance of their granules. In the stained preparations (fixation: Bouin) the acidophils of the rostral part contain rod-shaped granules — a surprising statement perhaps but nevertheless indisputable. Similar cells have been observed also in other species. In addition, these cells are less stainable than the caudal acidophils which contain normal granules. Fortunately, the specimens with persistent pars tuberalis are already histologically differentiated, and it could therefore be stated with certainty that the rostral basophilic region coincides with the area derived from the oral lobe in embryos. This distribution of the cells could be observed in 9 specimens of different sex and age, all present in my collection.

An entirely different type of pituitary is found in the *Geckonidae*, and we may choose *Tarentola delalandei* as an example (fig. 83: B). Also here the pars tuberalis is present as a few faintly violet cells in the brain wall. The pars intermedia is very large and consists of a single layer of columnar cells which have a high affinity to all common stains. After normal azan staining the granules appear deep purple or violet. They contain argyrophilic granules and seem to present the most aberrant type of all the cells in the adenohypophysis of this animal. The thin (caudal) part of the pars distalis, outside the hypophyseal cleft, contains a large proportion of distinct basophils, and in addition some acidophils and chromophobes. This caudal zone, characterized by the basophils, does not extend far rostrally. The rostral part of the pars distalis is characterized by very distinct acidophils, a few very pale and doubtful basophils and some chromophobes. I have not been able to distinguish more than one type of acidophils here.

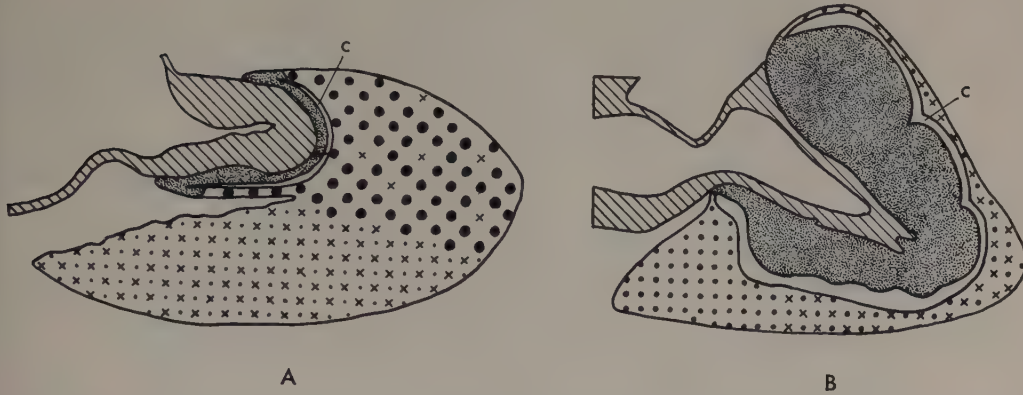


Fig. 83. Histological appearance of lizard pituitaries. The distribution of the acidophils (black dots) and basophils (crosses) is indicated. The intermedia is stippled, the neurohypophysis is striated. C = residual cleft. Only areas, not individual cells are marked.

A. *Anguis fragilis* (♂ ad., 18. VIII), median section. The dark acidophils (large dots) in the caudal part, the light acidophils (small dots) in the rostral part. Cp. figures 77 D and 84. Author's coll.

B. *Tarentola delalandei* (♀, Canary Islands, 27. VII). The acidophils are of one type (black dots), the basophils are present only caudally (crosses). Very large intermedia. Author's coll.

According to Atland (1939) there are no regional differences in the cellular distribution in the pituitary of *Sceloporus undulatus*. Atland distinguished basophils, chromophobes and acidophils, and believed that the two kinds of acidophils observed are different functional stages of the same cells. The intermedia cells of this species are distinctly acidophilic.

According to Miller (1948) the intermedia in *Xanthusia vigilis* stains a reddish purple, and the pars distalis is distinctly divided into two histological regions. The rostral half of the gland is characterized by a type of acidophils (type 2) with very fine granules, whereas the caudal half contains acidophils with coarser granules (type 1). Basophils and chromophobes are scattered uniformly.

In *Anolis carolinensis* there is also a distinct bipartition of the pars distalis according to Poris and Charipper (1938). The anterior portion of the gland contains basophils, chromophobes, and acidophils of the common type. The caudal portion consists mainly of non-granular cells with light red cytoplasm, which may represent a second type of acidophils. The intermedia stains red-purple.

Additional variations in the histological appearance of the lacertilian pituitary could be described. It may be mentioned here that the intermedia is decidedly acidophilic in *Varanus albigularis* and *V. niloticus*, both represented in my collection. The specimen of *V. albigularis* is remarkable also in other respects. There are at least two distinct types of acidophils. One of them has rod-shaped granules of the same type as mentioned in *Anguis*. As the fixative used in these cases was Bouin, the rods cannot be mitochondria. This peculiar type of acidophils is restricted

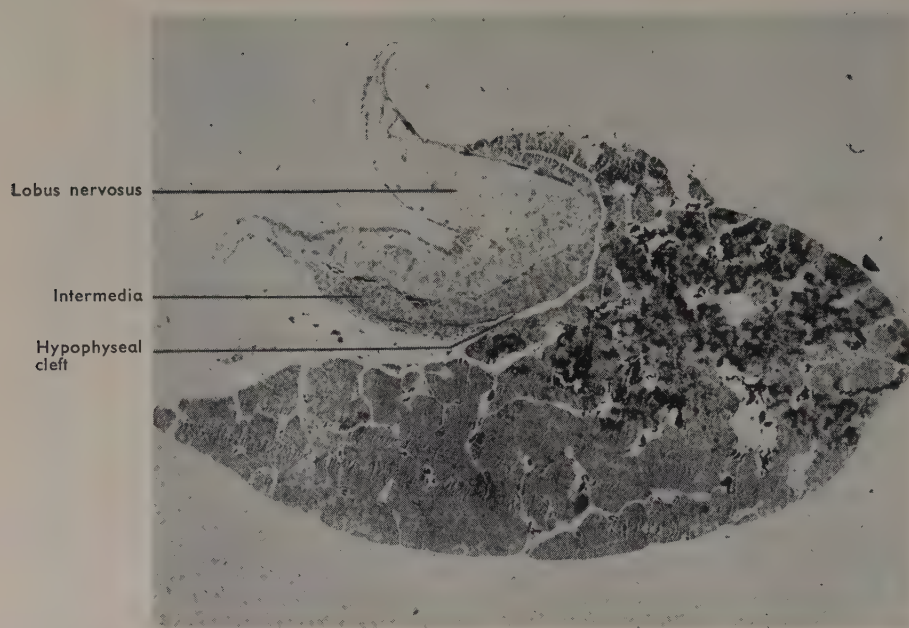


Fig. 84. The distribution of acidophils in the pituitary of *Anguis fragilis* (♂ ad., 18. VIII). The median section shows that the dark acidophils (black) are restricted to the caudal end of the pars distalis. The light acidophils and the basophils in the rostral end are not prominent in the photograph, nor are the basophilic intermedia cells. — Bouin, paraffin 3 μ , azan. Blue-green filter. 96 $\times$.

to the rostral part of the gland as in *Anguis*. The second type of acidophils has granules of the common type and stains more intensively with azocarmine. It extends a little further back than the first-mentioned type. The caudal part of the gland, in the neighbourhood of the pars intermedia, is made up of non-granular, faintly red cells and very few scattered basophils, and may correspond to the caudal region in *Anolis*. If the last-mentioned cells are regarded as acidophilic, we have three different kinds of acidophils in *Varanus*, all with different distribution. However, only one specimen of this species was available.

Trying to summarize the observations on lacertilians it can be said that the intermedia may show any kind of staining reaction but does not seem to be completely chromophobic in any lizard. As to the pars distalis the results from different species are highly divergent. As I have investigated a number of species myself and treated the preparations homogeneously (Bouin — azan) the differences must be real and not due to differences in the technique.

So, for instance, the basophilic cells may prevail in the rostral part of the gland (*Anguis*), they may, on the other hand, characterize the caudal part (*Tarentola*), they may be uniformly distributed, or they may be concentrated to a transversal belt in the mid-ventral part of the gland. The latter condition has not been

mentioned above but is present e. g. in *Varanus* and *Lacerta*. In late embryos of *Lacerta vivipara* the basophils are even exclusively restricted to a region just ventrally of the lateral lobes. Though there may be a certain correlation between the distribution of the basophils and the ontogenetic history of the gland in some species (*Anguis*, *Lacerta*, *Tarentola*) the site of the basophils is absolutely not fixed if the entire group is considered.

The same applies to the acidophils. In *Sceloporus* and *Tarentola* the acidophils are, as far as observed, uniformly distributed in the pars distalis. In other lizards there are at least two kinds of acidophils, one coarsely granulated, deep-staining and one with finer granules. The dark acidophils are, however, not always restricted to the caudal lobe as in birds. In *Anolis* and *Varanus albigularis* they are, in fact, present only rostrally.

The avian pituitary might be compared with that of such lizards as *Xanthusia* and *Anguis* in which the caudal part contains the dark acidophils, whereas the rostral part consists of less stainable acidophils and, in *Anguis*, basophils and chromophobes. This comparison is, however, extremely uncertain — we do not even know with certainty how to compare the acidophilic cells of different lizards.

Finally, the remains of the pars tuberalis in lizards have the same cellular appearance as in birds. Their plasm stains faintly violet in azan and I have found argyrophilic granules in some cells also in lizards. The pars intermedia of lizards will be discussed below.

Comparison with the Ophidia

I am also able to make a comparison with snakes, partly on the basis of good literature records, partly on my own material which contains 11 different species treated for histological purposes.

In *Thamnophis radix* the pars distalis is made up of basophils, small chromophobes and two kinds of acidophils (according to Sieler 1936). Nothing is mentioned about histological zones in the gland. The intermedia contains acidophilic cells of a columnar shape and, in the centre of the strings, faintly basophilic cells of irregular shape.

A distinct histological bipartition was found in this species by Cieslak (1945). The acidophils with coarse granules dominate the rostral end of the pars distalis whereas the pale acidophils together with chromophobes and basophils constitute the caudal half. Cieslak found a great individual variation in the staining properties of the pars intermedia.

Hartmann (1944) gives a detailed account of the histology of the pituitary in *Thamnophis sirtalis* and found distinct regularities in the distribution of the cells, which were of the same categories as those described by Sieler. The dark-staining acidophils (carminic cells) were largely restricted to the anterior half of the pars distalis throughout most of the year and were always absent from a posterior segment of the gland. The finely granulated cells (orange cells) were for the most

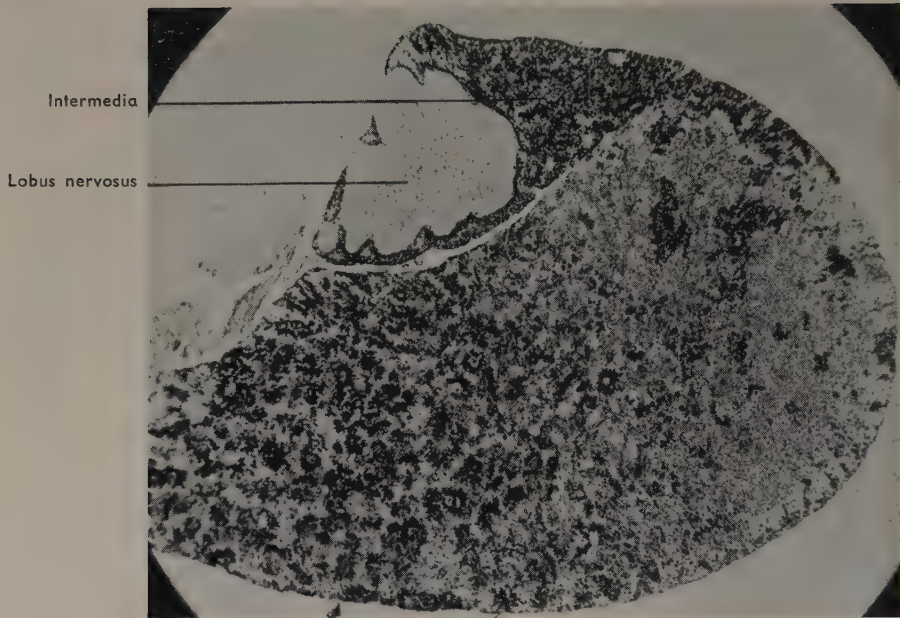


Fig. 85. The distribution of acidophils in the pituitary of *Tropidonotus natrix* (♀ ad., 4. VII). Approximately median section (the gland is asymmetrical). The dark acidophils in the rostral part of the gland and also the strongly acidophilic intermedia cells are stained black by the iron haematoxylin. The light acidophils in the caudal part of the pars distalis are not prominent in the photograph, nor are the basophils. Compare the pituitary of *Anguis* in fig. 84, where the distribution of the acidophils is just the reverse. — Bouin, paraffin 3 μ , iron haematoxylin of Heidenhain, acid fuchsine, phosphotungstic acid — aniline blue. 64 $\times$.

part limited to a posterior area in the gland, and the basophils tended to aggregate ventrally, near the site of gradual transition from orange to carmine cell preponderance. Some seasonal variations in the distribution of the cell types were observed, but this was the general pattern. The intermedia is said to consist of acidophilic cells and a few chromophobes.

In *Tropidonotus natrix* (several specimens) I have found a similar cellular pattern as that described for *Thamnophis* by Hartmann and Cieslak. The rostral half of the gland is dominated by the large, dark acidophils, which contain extremely distinct and large, globular granules (fig. 85). The granules stain with the haematoxylin of Heidenhain, with acid fuchsin, azocarmine and eosin. In addition, this part contains a few basophils, especially in its ventrocaudal part, and a variable number of chromophobes. The caudal end, near the intermedia, contains another type of acidophil with finer granules and little affinity to haematoxylin and azocarmine (orange cells of Hartmann). The basophils form a transversal belt across the ventral part of the gland in the transitional zone between the caudal and rostral acidophilic areas. In addition to the distinct basophils there are acidophils of both types and a few chromophobes here. The pars intermedia

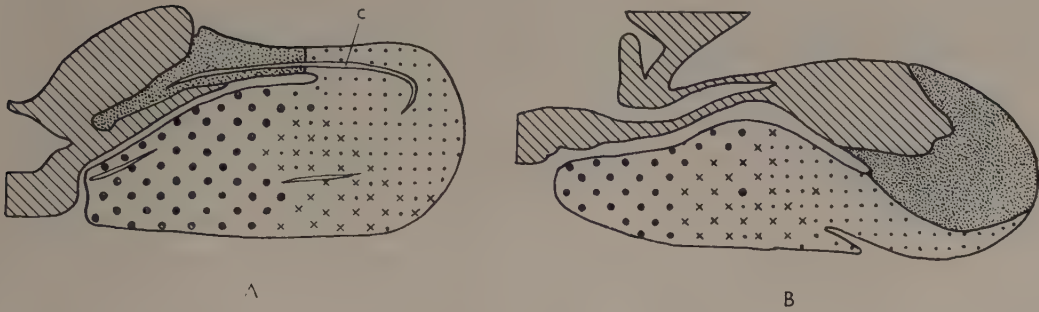


Fig. 86. Histological pattern of snake pituitaries. The dark acidophils (large black dots) are present in the rostral end, the light acidophils (small dots) in the caudal end, and the basophils (crosses) form a transversal belt across the middle. Intermedia dark stippled, neurohypophysis striated. C = residual cleft. Note, that only areas, not individual cells are indicated. Both these species have, in contrast to most snakes, a symmetrical pituitary.

A. *Bitis arietans* (ad.). Compare fig. 77 :C. Author's coll.

B. *Python reticulatus* (ad., in captivity). Author's coll.

contains heavily granulated cells, which stain distinctly in Heidenhain's haematoxylin, azocarmine and acid fuchsin. The granules are so compact that they cause considerable difficulties when the sections are cut. They are hard even if the gland has been fixed and embedded according to the freezing-drying process of Gersh (1932), a procedure which usually keeps the material very soft and easy to cut, since it does not imply any hardening in alcohol or other coagulating reagents.

In *Python reticulatus* (2 specimens, fig. 86: B) the rostral end is also occupied by large granular acidophils together with smaller chromophobes which occupy the central parts of the cell strings. The basophilic belt is very distinct here and contains in addition to the very bright basophils also chromophobes and acidophils. The caudal parts of the pars distalis contain mainly acidophils and chromophobes. The acidophils here are slightly different from the rostral ones but the distinguishing characters mainly concern the shape of the cells. The central parts of the strings contain, in all three zones, numerous large cells with both blue and red granules. The mixed character of these cells can be seen also after other staining procedures than azan. It may be mentioned that such cells are present also in some lizards. The cells of the intermedia are extremely chromophilic, deep red after azan staining, and the only additional cell type here is chromophobic or very indistinctly basophilic, and can be found in the centre of the strings.

In *Bitis arietans* (2 specimens, fig. 86: A), we find a similar distribution of the cells as in *Python* and *Tropidonotus*. The rostral end contains dark acidophils, chromophobes, and variegated cells of the type described for *Python*. The basophilic belt occupies an area near the posterior limit of the rostral acidophilic zone as in other snakes. It could be observed that this area continues into the epithelial stalk which was present in one of the specimens. The caudal end is very homogeneous and consists of a light acidophilic cell type with very fine granules. In fact, when the

granules in these caudal cells sometimes are absent or rare the cells get the appearance of chromophobes. The intermedia cells are acidophilic, a few cells in the centre of some strings appear to be chromophobic.

In general, the structure of the snake adenohypophysis thus seems to be characterized by the presence of two kinds of acidophils, which tend to occupy different ends of the gland. The rostral end of the gland, which is derived mainly from the processus anterior in embryos (p. 128), contains the coarse-granulated, heavily stainable acidophils, whereas a caudal area, evidently corresponding to the basal parts of the aboral lobe in embryos, is the site of the fine-granular, paler acidophils. The basophilic belt, which also contains acidophils of both kinds, probably corresponds to the central parts of the oral lobe. This latter conclusion is founded mainly on the fact that it is continuous with the epithelial stalk in one of the *Bitis*-specimens, and because of some observations in viper embryos. This position of the basophilic belt agrees well with the conditions in *Lacerta* and *Varanus* among the lizards. The intermedia is mainly acidophilic in all species. There is thus, in the species examined, a certain correlation between histological regions and the ontogenetic development of the gland.

Hartmann (1944) compared the snake pituitary with that of birds and pointed out that we have two kinds of acidophils in both classes. The site of the acidophils is, however, the reverse in birds, in which the coarse-granular ones are situated in the caudal part of the gland. Hartmann is evidently convinced of a fixed relation between histological regions and ontogeny and when comparing the development of the snake and the chick pituitary (as described by Baumgartner 1916, Rahn 1939, Payne 1942) he thinks he can find an explanation: "... it is evident that although the general developmental pattern is essentially similar, a slightly different series of growth shiftings occurs in the development of the hypophyseal complex in the two forms. As a result, the part of Rathke's pouch which will develop into the region dominated by carmine cells in the adult ultimately comes to lie in a more posterior position in the chick, and in a more anterior position in the snake. This embryological evidence suggests that the regions in which carmine and orange cells come eventually to predominate may be potentially different even at an early stage of development." I cannot see, however, that any figure in the papers of the said authors supports the hypothesis that the rostral end of the snake pituitary corresponds to the caudal end of the avian pituitary. In fact, there is some difference in detail concerning ontogenetic growth shiftings etc. in the two forms, but as shown in the preceding chapter the caudal lobe of the avian pituitary is derived from the aboral lobe. In the same way the aboral lobe forms the caudal part of the pars distalis in snakes, with the exception of the part forming the intermedia. The rostral part of the gland is derived from the oral lobe in both birds and snakes.

On the other hand, the great variability in the histological pattern of the adenohypophysis of lizards does not justify such strained attempts to find agreement of morphological and histological structure of the pituitary. We must therefore accept

the fact that the relations between morphological and histological structure *really are reverse* in birds if compared to the snakes. I may add also, that we do not know whether the A_1 -cells and A_2 -cells of birds correspond to the carmine cells and orange cells of snakes or not — as was apparently held by Hartmann.

Unfortunately, no histological comparison can be made with the Chelonia, Crocodilia and Rhynchocephalia, for the literature reports are too old and incomplete and my own material too scanty.

Comparison with Mammalia

In mammals the histology has been described well for a great number of species. For the present comparison it is of essential importance to consider the histological zones in the mammalian pars distalis, which have been described e. g. by Dawson and Hanström (cf. Dawson's review 1948 p. 103—105). A so-called zona tuberalis ("pars tuberalis interna"), lying in the pars distalis and continuous with the pars tuberalis, has been distinguished from the pars distalis proper in a number of species. The zona tuberalis is characterized by the total or partial absence of acidophils and consists mainly of chromophobes and basophils. In the whales and the elephant it is very distinct and delimited (Jacobsen 1941, Hanström 1944, 1946), in carnivores and some other mammals it is more diffuse or not clearly recognizable (cf. Hanström 1946 a, 1947, Dawson 1948, Romeis 1940). It is also evident that it may occupy a considerable part of the gland, as in the cat, or have a much more restricted extension as in the whales.

It has been suggested, yet with due reservation, that this zona tuberalis in mammals corresponds to the less stainable, caudal lobe in birds (Rahn and Painter 1941). We shall try here to answer this question from a morphological and histological point of view.

As shown in the preceding chapter the oral lobe of Rathke's pouch and the basal parts of the lateral lobes, if included in the pars distalis, occupy a varying position in relation to the pars tuberalis in the adult mammal. Either the derivatives of the oral lobe are situated near the very rostral end and form a direct continuation of the pars tuberalis (rabbit, rat etc.) or, these parts of tissue become embedded in the ventral surface of the gland (cat). In the latter case they are connected with the base of the pars tuberalis proper by strands of tissue along each side of the pars distalis: the basal parts of the lobi laterales. It is now essential to know whether these vestigial morphological units correspond to the histological "zona tuberalis" or not, and we may choose a few examples to illustrate this question.

In the *rabbit*, described histologically by Dawson (1937), the zona tuberalis extends along the ventro-median surface of the pars distalis from the base of the pars tuberalis almost to the caudal end of the gland (fig. 87: A). Comparing fig. 80 we may conclude that the very rostral part of the zona tuberalis corresponds to the cephalic lobe of birds (oral lobe in embryos), but that a considerable part of the zona must be derived from the aboral lobe. The dislocation of the epithelial

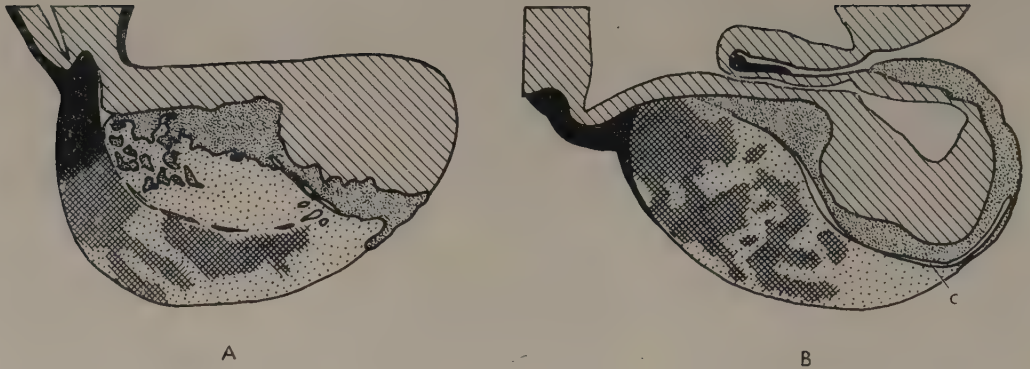


Fig. 87. Histological regions in the mammalian pituitary. Light stippled = pars distalis, dark stippled = pars intermedia, cross-striated = zona tuberalis, black = pars tuberalis, obliquely striated = neurohypophysis. C = residual cleft.

A. Rabbit (*Oryctolagus cuniculus*), median section of the pituitary. After Dawson (1937, fig. 1).

B. Cat (*Felis domestica*), median section. Coll. Hanström, Lund. The zona tuberalis is only roughly indicated because it was not very distinct in this specimen, and was partly adjusted so as to agree with Dawson's descriptions and figures (Dawson 1937, fig. 10—17).

stalk and the lateral lobes to the extreme rostral end, which can be followed in the rabbit embryos, definitely shows that the oral lobe must be restricted to a very small rostral area in the adult.

The conditions stand out more clearly in the *cat*, described by Dawson in the same paper (1937). According to the embryological investigation, there is originally no continuity between the base of the pars tuberalis and the very rostral end of the pars distalis (fig. 82: B). And therefore the following statements of Dawson's (p. 478) are not astonishing: "In the cat the typical tuberalis tissue is sharply delimited from the zona tuberalis while in the rabbit one gradually merges into the other." Undoubtedly, the gradual transition from the pars tuberalis to the pars distalis is mediated by the remnants of the oral lobe in the rabbit. And the sharp limit in the cat is explained by the fact that there is no direct continuity originally between the rostral end of the pars distalis and the pars tuberalis in this species. Further Dawson has clearly described how the typical tuberalis in the cat continues as short strings, one on each side, along the surface of the pars distalis. These strings are situated exactly so as to correspond to parts of the lateral lobes in fig. 82: B. They were distinct also in the specimen drawn in fig. 87: B. Dawson does not mention any structure, however, which could correspond to the oral lobe. The zona tuberalis in the cat is thus mainly made up of the modified area of the aboral lobe. Probably also the remnants of the oral lobe and the very basal parts of the lateral lobes are included. The zona tuberalis of the rabbit, on the other hand, consists of the modified area of the aboral lobe and of the oral lobe. Whether the lateral lobes take part in the formation of the zona or not depends on the choice of limits for the zona tuberalis in this species.

The zona tuberalis of mammals is thus a complex structure, and it is far from certain that it can be regarded as homologous throughout. If the oral lobe and the bases of the lateral lobes take part in the formation of the zona tuberalis, these parts may be homologized with the equally developed parts in other mammals. The main part of the zona tuberalis is, however, a modified area of the aboral lobe as in the two species considered above. Concerning this part we have but slight support for homologies, although the position indicates that its origin may be approximately the same in different mammalian species. Dawson pointed out (1948) that the branches of the portal veins are primarily distributed within the zona tuberalis. Perhaps this is a reason for the characteristic histological appearance of this part, for the portal blood seems to have a powerful influence on the glandular cells of the pars distalis (Dawson 1948). The question will then be whether we can use the distribution of the portal vessels as a criterion for homology between the parts called zona tuberalis in different mammals or not. If we turn to other animal groups the portal vessels have a rather indefinite area of distribution within the pars distalis. In adult birds, for instance, the portal vessels spread directly into the caudal and cephalic lobes as soon as they reach the surface of the gland, whereas the lateral lobes continue far into the gland as a pars tuberalis interna on each side. In birds the portal vessels are thus not restricted to any definite morphological part of the adenohypophysis nor do they enter the gland along the basal parts of the lateral lobes. They can therefore hardly be used as criteria for the homology of the main part of the zona tuberalis.

If Dawson's (1948) interpretation is correct and the humoral influence of the portal blood thus governs the differentiation of the zona tuberalis, the latter must of course be rather independent of morphological homologies. It may, of course, even then contain homologous elements but its extension within the gland is no longer morphologically defined. However, it should be pointed out that such an area of basophils and chromophobes is not produced around the entrance of the portal vessels in the pituitaries of all amniotes. Even some mammals seem to form an exception, viz. those without a zona tuberalis. I found in injected specimens that the portal vessels of snakes enter the very rostral end of the gland — that is, they enter the most acidophilic part of it! And in birds the portal vessels are distributed partly within the cephalic lobe with its more chromophobic and basophilic appearance, partly within the caudal lobe with its A_1 -cells. However, since the physiological and histological differences between mammals, snakes and birds are so great, these facts cannot be used as evidence against Dawson's hypothesis.

To sum up: The zona tuberalis of mammals is a complex structure. Its morphological significance is often difficult to find out in adult material and is not clearly revealed by its histological structure. The term may, of course, be maintained for the histologically differentiated area continuous with the pars tuberalis and characterized by a total or partial absence of acidophils, but it should be remembered that the term is used for a complex of different morphological elements, some of which may be lacking:

1. A modified area of the pars distalis, derived from the aboral lobe (the main part).
2. The vestigial oral lobe.
3. The basal parts of the lateral lobes which may fuse with each other or with the pars distalis.

Now the problem of possible homologies between the zona tuberalis of mammals and the cephalic lobe of the avian pituitary can be discussed. It is evident from the preceding discussion that *the cephalic lobe in birds does not correspond to the main part of the zona tuberalis of mammals* — viz. the part derived from the aboral lobe — *but only to the part which represents the vestigial oral lobe*. If the basal parts of the lateral lobes contribute to the zona tuberalis, as they probably do in the cat they are of course directly comparable to the “pars tuberalis interna” in birds.

Here I wish to make a remark on the terminology. The term “pars tuberalis interna” has been used in this paper for the bases of the lateral lobes when they have been included in the pars distalis. I think the term is quite adequate in this sense, as it is used for a part of the pars tuberalis which has been included in the main gland. Its morphological significance is quite clear. The term “zona tuberalis” could then be used for the said histological region in mammals and only refers to a zone with a definite cellular structure, but with uncertain and perhaps varying morphological significance.

The original hypothesis of Rahn and Painter (1941) that the cephalic lobe of birds and the zona tuberalis of mammals should be homologous was set forth because of a similar histological appearance of these regions. Further, we have concluded above that this was partly true, because the oral lobe, in some mammals at least, is included in the zona tuberalis. It would thus appear that homologization from histological criteria had been successful in this case. However, it appears to me that the similar histological appearance of these homologous areas is merely accidental. A comparison between birds and mammals is always something like a short circuit, since these groups are related only so far as they originate from some common reptilian ancestors. The present-day reptiles have, of course, also changed considerably since the time of these ancestors, but still they inform us about the potencies for variations hidden in the old reptilian stock. Comparisons between birds and mammals could therefore hardly be made without considering also the reptiles. If we turn to the present-day reptiles we find extensive variation. The part homologous to the avian cephalic lobe may be largely basophilic (*Anguis*), it is acidophilic (*Varanus*, snakes) or, it is less stainable (*Lacerta*, *Xanthusia*). There were thus several possibilities in the old reptilian stock, and the fact that birds and mammals show some similarities may either be accidental or may depend on some recently acquired physiological conditions (e. g. humoral influence). If the latter is true we have still less reason for using the histological similarities as criteria for homologies.

The Pars tuberalis and the Pars intermedia

The preceding discussion has mainly dealt with the pars distalis. A few lines will now be devoted to the pars tuberalis and the pars intermedia.

In contrast to the great variability of the pars distalis the pars tuberalis, when present, has a remarkably constant histology in all amniotes, although it is represented only by a small vestige in some of them. Its absence in snakes and some edentate mammals (Wislocki 1938) indicates that it cannot have any fundamental function in the amniote vertebrate, and its vestigial state in many animals (lizards, some birds, some mammals) supports this view. The indistinct granulation in its cells also seems to agree with this supposition. On the other hand, my investigations on birds and reptiles make me believe that it really has a function, as soon as it is present. Both in lizards and birds some cells in the pars tuberalis are packed with argyrophilic granules, and such a differentiation can be observed also in very young embryos. This granulation which is highly specific, together with the colloid acini in many animals, certainly indicates a secretory activity in the pars tuberalis. The conclusion must therefore be, *that the pars tuberalis is functional whenever present, but that its function cannot be essential for the maintenance of the amniote organism*. The nature of this function can hardly be predicted theoretically. Experiments with the frog *Xenopus* (Hogben and Slome 1936) indicate that a factor antagonistic to the melanophore-expanding hormone of the pars intermedia is produced by the pars tuberalis or by some associated structure in this animal (confer also Atwell, 1938).

It has been suggested (Harris 1947) that the pars tuberalis should serve to establish the link of portal vessels between the brain and the pars distalis. Though this hypothesis appears to be well founded and may give some explanation to the curious appearance of the pars tuberalis, my embryological investigations have given little support to it (chapter XIII).

The pars intermedia. There can be no doubt that some parts of the avian pars distalis which are derived directly from the top region of Rathke's pouch correspond morphologically to the pars intermedia of reptiles and mammals. No discrete histological region is, however, formed by this primordium; the reasons are briefly discussed on p. 117. It is thus merely a question of nomenclature if there is an intermedia in birds or not. Most authors deny its presence in birds, and this is certainly most reasonable.

A few general considerations on the intermedia are, however, of interest in this connection. What is understood by the concept intermedia? Atwell defined it developmentally in his ontogenetical study on the human pituitary (1926) as "that portion of the wall of Rathke's pouch which early comes into contact with the neural lobe, remains relatively thin and epithelium-like, faces the residual lumen, and does not become strongly vascularized". This definition applies to the embryonic intermedia of mammals and many other animals, but cannot be used for the adult pituitary of many species. On the other hand, it also applies to the

"intermedia" of avian embryos, but Atwell (1939) and most other authors do not attribute any intermedia to adult birds.

Is it possible to define the intermedia of amniotes unmistakably on a morphological basis? In fact, this seems to be difficult, for it is uncertain whether it is constantly formed by the same wall of Rathke's pouch. In snakes the intermedia is formed by the top region of Rathke's pouch, including parts of the rostral and caudal walls (fig. 77: A). In man it is formed by the caudal wall (Atwell 1926), and in the cat it seems to be formed by the rostral wall if I interpret Brahm's figures (1932, fig. 2—5) right. The significance of such differences is of course doubtful. The top of the pouch may "wander" so that cells originally present on the rostral side may appear on the caudal side etc. The differences therefore do not exclude that the intermedia is homologous throughout. But the embryological criterion is consequently uncertain and not sufficient for an exact definition. Perhaps any wall of the aboral lobe can form an intermedia in some animals, provided it is brought in a certain relation to the neural lobe. The residual cleft cannot be used in a definition of the adult intermedia for it is entirely absent in many adult snakes and some Primates (Hanström 1948), and in snakes it has an aberrant situation already at early embryonic stages. The vascularization of the intermedia is usually less developed in mammals and some reptiles, but is fairly rich in some reptiles. The intermedia may proliferate and constitute a considerable part of the gland in many reptiles (some snakes, *Tarentola*). These last-mentioned characters can therefore hardly be used for a general definition.

It is also very difficult to find any histological feature, diagnostic cells, or cell arrangement which could be used for a definition. In mammals the intermedia cells are chromophobic or faintly basophilic, but in different reptilian species we find strongly basophilic, acidophilic, highly chromophilic, or faintly staining cells in the intermedia, even if the technique has been entirely uniform (e. g. *Anguis*, *Python*, *Tarentola*, *Lacerta*). The morphology of the cells differs to the same extent. The reason why investigators distinguish an intermedia is that this zone differs histologically from the pars distalis proper, and that it is attached to the neural lobe. Further the ontogenesis must be taken into consideration for a definition, and it is necessary to exclude the non-specific chromophobic strings in birds (cf. p. 71) from the concept. A definition of the intermedia in amniotes would then be:

A zone of epithelial tissue, in contact with the neural lobe, derived from the top region of the Rathke's pouch, and mainly made up of specific cells, which cannot be refound in the pars distalis proper.

This definition is mainly histological and does not state definitely whether the intermedia in different animals is homologous or not. Though this seems probable the embryological and structural criteria give slight support, and only the relation to the neural lobe remains as a positive evidence.

In spite of its extremely varying appearance the intermedia seems to have the same function in all animals, viz. the secretion of the melanophore hormone. This hormone is present in the pituitary in all vertebrates (cf. Oldham, Callonway, and

Geiling 1941, Kleinholtz 1938, Witchi 1937). Its exact site of formation has been localized in a number of mammals, snakes and amphibians to the pars intermedia (cf. Oldham, Callonway and Geiling). Considering the vast histological difference between the intermedia of mammals and snakes it is safe to state that the cells which produce the melanophore hormone may look widely different and do not seem to have any specific histological features in common. This is still more striking if the melanophore hormone of the lacertilians turns out to be produced by the intermedia. In birds the melanophore hormone is produced mainly by the cephalic lobe — not by the caudal lobe which contains the morphological equivalent of the intermedia (De Lawder, Tarr and Geiling 1934, Kleinholz and Rahn 1940). Also in some mammals with completely or entirely reduced intermedia the hormone seems to be formed in the rostral part of the gland (e. g. in the armadillo and whale, Oldham, Last and Geiling 1940, and in man, Jores and Glogner 1933). The embryological investigation indicates that the production of the melanophore hormone is associated with the Ag-cells in birds, and this agrees with the statement that the typical Ag-cells are most common in the cephalic lobe, where the hormone is formed.

Correlation between Function and Morphological Homologies in the Pituitary

Finally, we may discuss the problem, whether morphologically homologous parts of the adenohipophysis have the same function in different animals or not. As mentioned above it is fully evidenced that the melanophore hormone can be produced in non-homologous parts of the pituitary in different animals. In birds its site of formation is mainly the cephalic lobe (the oral lobe in embryos), but it is the intermedia (top of aboral lobe) in snakes and most mammals. Further, if the basophils have a similar function in all amniotes, this will mean that there is very little correlation between morphological homologies and function. I repeat that the basophils are restricted to the anterior end, to the caudal end, to a transversal midventral belt, or are uniformly scattered in different species of reptiles. And since there is no general histological pattern which applies to amniotes in general, not even approximately, great differences in the localization of the functions of the pars distalis within the amniotes must be assumed. On the other hand, the histological appearance of the pituitary in birds, mammals and snakes seems to be very uniform within each of these groups. This might indicate also that the functions have a fairly constant site in the pars distalis of the members of the respective classes. The great variability within the lacertilians, on the other hand, indicates great differences in the functional pattern of the pituitary in this group.

Realizing that the mammals and the birds have arisen independently from reptilian ancestors and that the histological pattern of the reptilian pituitary may vary extremely, it is only natural that the adenohipophysis of birds and mammals is difficult to compare histologically.

It might be suggested that migration of cells within the hypophyseal anlage during the ontogenetic development might cause this peculiar histological variation. This would mean that the cells with identical staining reaction originate in the same area of Rathke's pouch but later become dislocated. So long as Rathke's pouch is single-layered with columnar epithelial cells in the wall such a migration is out of the question. And the mode of proliferation which is characteristic of the hypophyseal anlage — the formation of buds pointing more or less perpendicularly to the single-layered original wall — does not support such a hypothesis. Further the histological zonation of the gland appears so early (cf. chapter IV) that proliferation has hardly begun and the hypothetical migrations can hardly have taken place. The final proof against such a suggestion is, however, more quantitative. In order to explain the reverse conditions in snakes and birds by the "migration" hypothesis it must be assumed that, in one of the forms, most cells in the rostral half of the gland have migrated to the caudal half and the cells there have migrated in the opposite direction. I do not think this intensive traffic could take place without visible disarrangement of the anlage. Of course this does not mean that no mixing of material of different origin occurs. Along the border lines where oral and aboral lobe have fused in birds, a mutual invasion of material may perhaps occur and may be the reason why some very distinct border lines show irregularities and become less distinct at places. However, this concerns a narrow zone only and has little to do with the almost total inversion of cellular material required to explain the diversity of histological pattern within amniotes.

CHAPTER VII

Colloid Cysts, Lymphoid Tissue, Parasites and Other Exceptional Structures in the Avian Pituitary

Colloid Cysts

The colloid cysts in the avian pars distalis have been observed by several authors and were described particularly by Collin (1926), Voigt (1939), Rost (1939), and Obussier (1948). As the cysts are so frequent that they may be regarded as a quite normal feature in the gland they will be described more in detail. In order to define the term I have classified as cysts all colloid-filled bladders which exceed $50\ \mu$ in diameter and further all colloid masses surrounded by an epithelium different from the common glandular tissue (disregarding size). The common colloid acini (cp. p. 39) are thus excluded from the concept.

Obussier (1948) found cysts in about 57 % of his fowl pituitaries and in about 43 % of the domestic ducks. He did not find any cysts at all in the wild ducks and made it probable that the frequency of cysts increases when the animal is domesticated. Even if this statement is true — and the facts supporting it are strong — it is unquestionable that the cysts are very common also in wild birds. In fact, 77 pituitaries out of 142 in my collection contain one or more cysts, and the domesticated specimens in this material are very few. I have also found ciliated cysts in the pars distalis of a wild duck (*Anas platyrhynchos*), so that the absence of these in Obussier's material of wild ducks must certainly be accidental, even if his opinion of the influence of domestication on the frequency of the cysts is right.

My material tends to show that the cysts are more frequent in large birds than in small ones, but they are present both in one of the small humming-birds (*Sephanoides*) and in an albatross (*Diomedea melanophris*). The distribution of the cysts within the pars distalis is not uniform, but there is a distinct concentration in the region near the attachment of the epithelial stalk. Cysts may, however, occur in all parts of the caudal and cephalic lobes and I have even found several large ciliated cysts in the pars tuberalis of a specimen of *Coragyps atratus*.

In most cases there are only one or a few cysts in the same gland, and more than 5 cysts are rarely recorded. Yet there are 20 cysts in the pars distalis of a pheasant (*Phasianus colchicus*) and about 25 in one specimen of *Pica pica*. It should

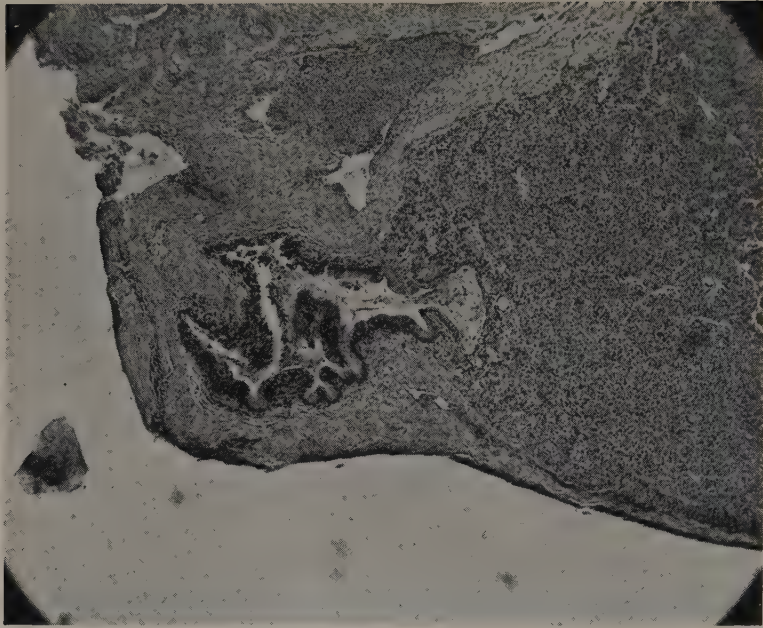


Fig. 88. Cyst in the pars distalis of a hooded crow (*Corvus corone cornix*, ♀ juv.). The cyst has extended through the capsule into the surrounding connective tissue. It has probably collapsed when the gland was dissected out. — Susa, paraffin 6 μ , haematoxylin of Ehrlich. 80 $\times$.

be noted that the identification of the colloid masses as cysts in these cases is definite because the surrounding walls are partly ciliated.

The size of the cysts, as expressed by their maximum diameter, usually varies between 20 μ and 500 μ , but in extreme cases they may reach a considerable extent and bulge out from the surface of the organ (fig. 88). An exceptionally large cyst in the caudal lobe of *Phalacrocorax magellanicus* is shown in fig. 89. Here the cyst is situated near the posterior end of the gland and extends far behind it into the hindmost part of the sella embracing the intercarotic anastomosis. This case as well as a similar large cyst in the lateral wall of a black-cock pituitary (*Lyrurus tetrix*) are certainly to be regarded as pathologic, whereas the common cysts may be regarded as more normal structures.

The cysts are filled with a colloidal matter which is sometimes hardly visible in the azan preparations, sometimes stains a deep blue. It may contain formed bodies of different size which either stain red or blue in azan. Also the lining epithelium varies considerably in appearance. It is always single-layered and, in the majority of cases, consists of cubic or flattened cells which usually carry cilia on their free surface. Otherwise the epithelium varies within wide limits: it may be ciliated or not, the cells may be high columnar, cubic, or extremely flattened so that they are difficult to distinguish. The plasma of the cells stains reddish in azan and, if cilia are present there is a dark margin along the ciliated surface of the cell

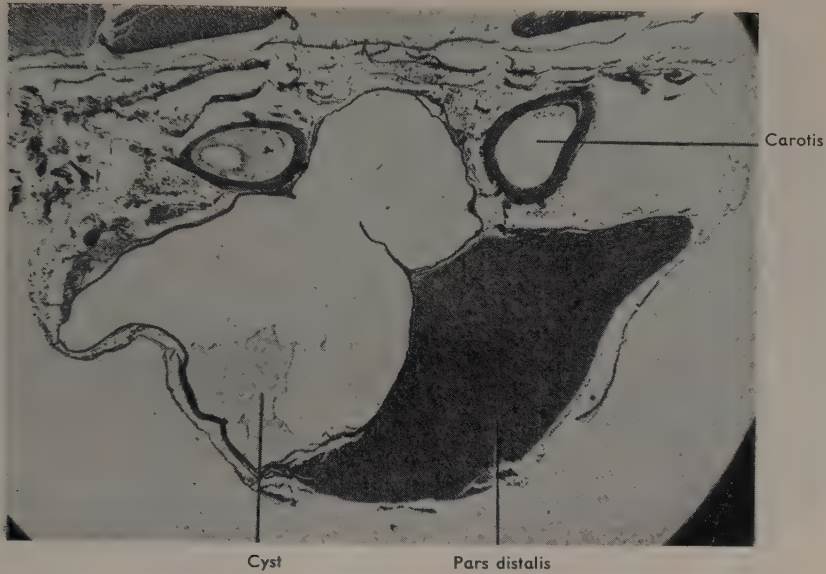


Fig. 89. Large cyst in the caudal lobe of the pars distalis of a cormorant (*Phalacrocorax magellanicus*). Transversal section through the posterior end of the gland. — Bouin, paraffin 8 μ , azan. 29 $\times$.

marking the row of basal bodies. The ciliated epithelia frequently contain scattered blue-staining glandular cells of the same appearance as the mucus-cells of the pharyngeal epithelium. In addition, degenerating cells of different kind are often observed; they are hyperchromatic, red, or are packed with blue-staining colloid. Their remnants probably give rise to the formed bodies in the colloid of the cysts. All the different kinds of epithelia may be found in different cysts of one and the same gland (fig. 90).

A common feature is that the epithelium becomes lower in the very large cysts. Sometimes it is even difficult to distinguish the narrow zone of plasma belonging to the epithelial cells which have approximately the same shape as the endothelial cells of blood vessels. It is therefore difficult to confirm Collin's statement that some cysts are surrounded exclusively by connective tissue. It is easily observed, however, that the walls of the larger cysts are supported by a thick membrane of connective tissue whereas such capsules are only faintly developed around the small cysts.

Voigt (1939) obviously regards the mucus-secreting cells in the ciliated epithelia as ciliated cells in the state of disintegration. As they are, in many epithelia at least, almost perfectly like the mucus-secreting cells of the pharynx and rarely show any signs of degeneration they must certainly be regarded as a permanent cell type distinct from the ciliated cells (fig. 90 A). This view is also shared by Rahn (1939, p. 494).

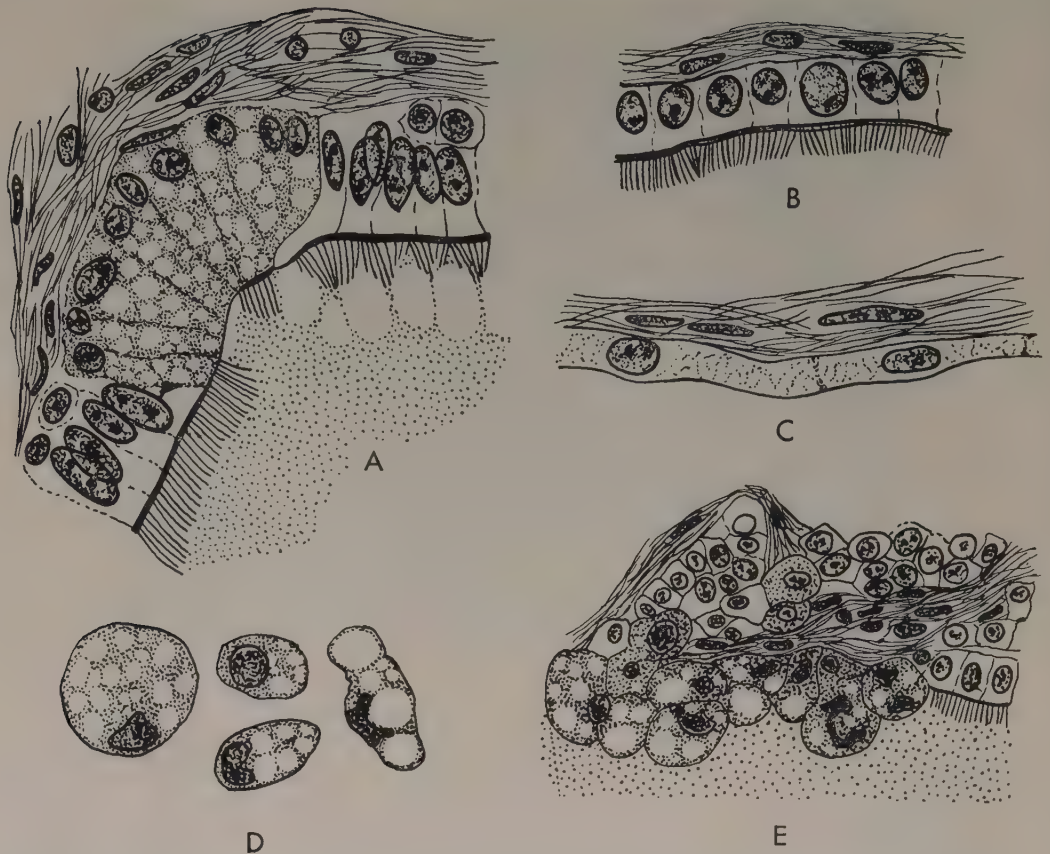


Fig. 90. *Somateria mollissima*, ♀ ad. The structure of different cysts in the pars distalis. A. Columnar, ciliated epithelium containing a group of mucous cells (vacuolar, in the preparation blue). B. Cubic, ciliated epithelium. C. Flattened, non-ciliated epithelium. D. Suspended cells in the colloid of a cyst. E. Cells identical with those in D being formed in the wall of a cyst. — All figures from 6μ sections, stained in azan. A—D $1100\times$, E $700\times$.

The disintegration of cells in the walls of cysts results in the presence of cell fragments and even whole cells in the colloid. In some cysts these suspended cells are very numerous, especially in an eider-duck in my collection (fig. 90). The suspended cells are sometimes very fragmentary, but in some cases they show the same characteristics as the cells described in the "Rathke's cysts" of *Homo* by Romeis (1940) and in *Cercopithecus* by Hanström (1948). They have wrinkled and irregular nuclei and a vacuolar slightly bluish plasm. Romeis regards these cells as degenerating glandular cells which have entered the cysts and are packed full of lipoids before they finally disintegrate. In my specimen of *Somateria* the degeneration of these cells has begun before they leave the wall of the cysts, and in fact some walls seem to produce such cells in a way similar to holocrine secretion (fig. 90 E).

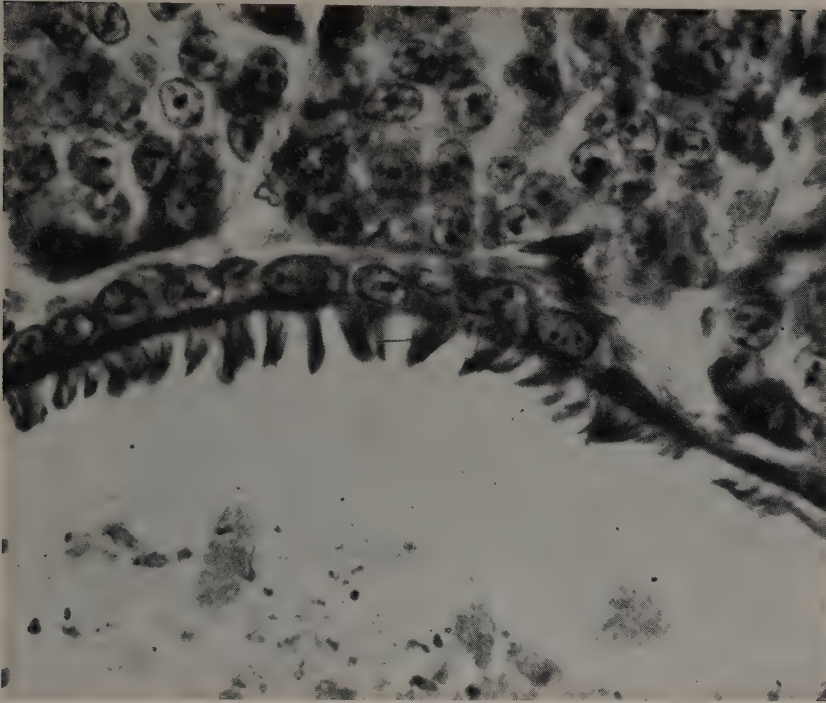


Fig. 91. Ciliated epithelium in a cyst in the pars distalis of a goose (*Anser anser*, ♀). — Bouin, paraffin 8 μ , impregnation according to Bodian and over-gilding. 1400 $\times$.

There is some disagreement in the literature concerning the ontogenetic origin of the cysts. Voigt (1939) stated that the cysts in the chick embryo arise in connection with the remnants of the residual lumen of Rathke's pouch, but Rahn (1939) could not state such a development. My preparations indicate that cysts may arise in three different ways, and the disagreement in the literature may therefore be imaginary.

1. *Rathke's cysts* or cysts arising in connection with the residual lumen, will be described first, and I choose chick embryos to illustrate what happens. The lumen of Rathke's pouch is practically continuous until the 6th day of incubation, but is later subdivided into smaller clefts and bladders. In the gland proper the oral and aboral lumina are often distinct and large for a long time, but in the epithelial stalk and adjacent part of the cephalic lobe there are usually many small bladders with very small and cleft-like lumina. These bladders are often directly connected with parts of the original lumen. They are surrounded by an epithelium which differs from that of the cephalic lobe already in the 10-day embryos. The cells are indistinctly delimited, and their plasm is light red and homogeneous in azan preparations. In the 14-day embryos these cells have acquired

cilia, and it may be noted that also Rahn (1939) and Voigt (1939) found cilia for the first time in embryos of this age. Two days later also the mucous cells are distinct. This process, leading to the formation of cysts from parts of the original lumen and characterized by the early appearance of ciliated cells in the walls has only been observed in the ventral part of the cephalic lobe near the epithelial stalk. As the cysts in adult birds show a distinct concentration to this region they have probably often arisen in this way. The remnants of the oral and aboral lumina are surrounded by common pituitary cells in the embryos. Even if colloidal matter is often deposited in these lumina and they assume a bladder-like shape in some cases there is not much to support the view that they should give rise to ciliated cysts. The ciliated cysts in the dorsal parts of the gland and in the caudal lobe must probably have another origin, presumably the one indicated below.

2. *Cysts arising by disintegration of large mucous cells.* Rahn (1939) observed large isolated mucous cells in the middle of cells cords of embryonic glands. He suggested that these cells may form the centre of future ciliated cysts. I have also seen a few of such large cells, sometimes a few together and near cells similar to the prospective ciliated cells described above. Though it is difficult to state that cysts do develop in this way this possibility must be left open.

3. *Cysts arising from colloid acini.* In my specimens of *Lyrurus tetrrix* there are several large cysts, particularly in the zone near the neural lobe (fig. 40). The walls of the cysts are single-layered, and their cells are not very different from those in normal tissue. Since all transitional stages from the common small colloid acini to such cysts can be found their origin is quite clear. It is difficult to decide whether cysts often arise in this way.

The great variability of the cysts and their absence in about half my specimens show that they are not necessary for the function of the pituitary. On the other hand, they can hardly be regarded as pathologic, since they occur so frequently and obviously are harmless. Some cysts, e. g. the one in fig. 89, may be classified as pathologic, because they must mean a menace to the function of the gland and have destroyed parts of it. In most cases, however, they are certainly physiologically indifferent and may develop either by an exceptional deposition of genuine hypophyseal colloid (as in the black-cock) or by the action of ciliated and mucous cells which must be regarded as foreign elements. The latter kinds of cells undoubtedly indicate an extension of the potencies governing the differentiation of the pharyngeal epithelium along the hypophyseal stalk into the pituitary proper (cf. Voigt 1939, Rost 1939), and if the mucous cells contribute to the content of the cysts this must certainly be a "mucus" of the same kind as in the respiratory parts of the pharynx. It is only natural therefore, that ciliated epithelia and mucous cells are most common in the region near the epithelial stalk, i. e. the part nearest the oral epithelium.

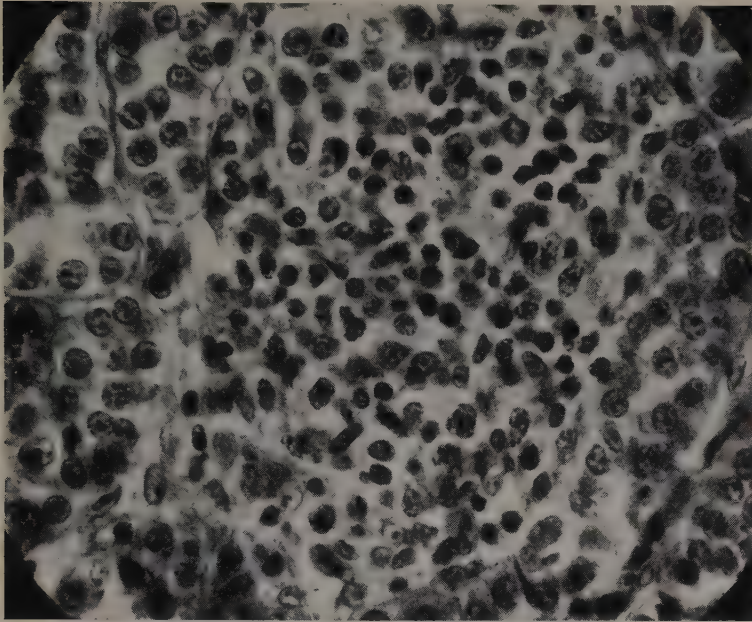


Fig. 92. Lymphoid tissue in the pars distalis of a *Larus argentatus* (♀, one year old). Note the normal tissue to the left and to the right. — Bouin, paraffin 8μ , azan. $770\times$.

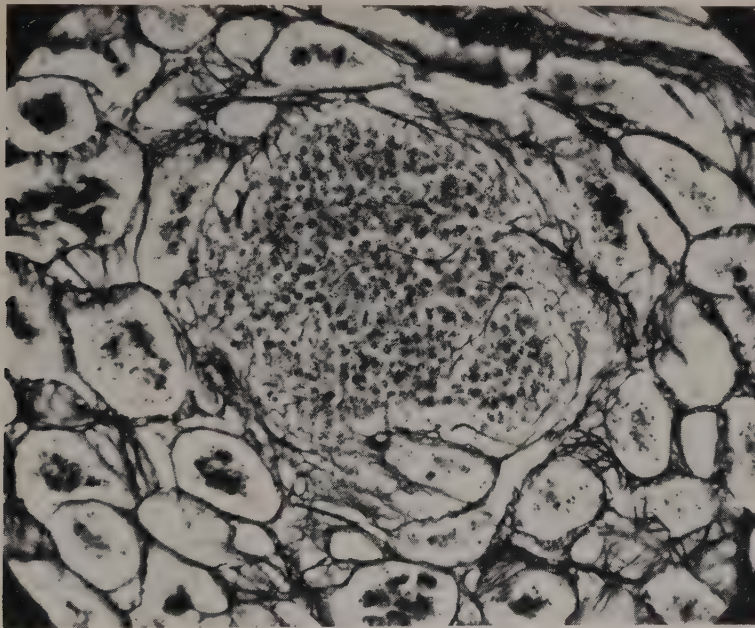


Fig. 93. Lymphoid nodule in the pars distalis of a goose (*Anser anser*, domestic, two years old). The nodule is sharply delimited from the normal tissue. — Alcohol-formalin-acetic acid, paraffin 10μ , impregnation of reticular fibres according to Gömöry. $340\times$.

Lymphoid Tissue and Other Exceptional Structures

Lymphoid tissue has been found in the pars distalis of 22 birds out of 111 and thus is not very rare. The nodules are often small — less than 100μ — but in one specimen of *Diomedea epomophora* a very large nodule extends throughout the region of junction between the portal zone and the pars distalis. It should be noted that the lymphoid cells are typical with small dark nuclei, and no confusion with the heaps of chromophobic glandular cells was possible (fig. 92). The lymphoid islands lack "germ centres" and are sometimes very sharply delimited from the surrounding epithelium, sometimes more diffuse. Gömöry impregnation shows that the rich system of reticular membranes present elsewhere in the gland is absent in the nodules where only scattered argyrophilic fibres are seen (fig. 93).

It is interesting to note that lymphoid tissue seems to be quite absent from the human pars distalis whereas it occurs in the pars intermedia (cp. Romeis 1940 p. 363). In birds, however, where the intermedia is absent, the lymphoid tissue occurs in the pars distalis. Most lymphoid islands were found near the entrance of the portal vessels in the cephalic lobe, only four were clearly situated in the caudal lobe.

Obussier (1948) found "Hyperplasien" in 6 out of 66 examined fowl pituitaries. I have not seen any hyperplastic epithelial tissue at all in my specimens. As my material mainly consists of wild birds this statement may support Obussier's theory that such alterations are due to domestication.

Some other exceptional structures were observed in a few specimens. I may mention the presence of a diffuse fibrosis in the pars distalis of an adult mag-pie and the presence of bulb-like bodies of squamous epithelial cells in the vestigial epithelial stalk and adjacent parts of the pars distalis of an old hen (*Gallus*). The latter case is interesting if compared with the occurrence and development of such islands of squamous epithelium in man (Erdheim 1904, Romeis 1940 p. 272). They are present only in the pars tuberalis of adult human pituitaries but are said to be derived from the epithelial stalk which is later included in the base of the pars tuberalis. The presence of these structures just in the epithelial stalk of the old hen is therefore probably not an accidental incidence.

Parasites

Parasites have been found in a few cases. Large megaloschizonts of the haemsporidian *Leucocytozoon sakharoffi* Sambon were found in the pars distalis, in the neurohypophysis and in the hypophyseal capsule of a young crow (*Corvus corone cornix*) (Wingstrand 1947). Later I have also seen a megaloschizont of *L. ? berestneffi* in the pars distalis of a mag-pie (*Pica pica*).

Nematodes have been reported from the pituitary of the cat (Widakowich 1905) and from the dog (Obussier 1941). In my material nematodes have only been found

in the pituitary of two birds, both adult wild ducks (*Anas platyrhynchos*, ♂ and ♀, Lake Hornbergasjön, ³¹/₅ 1945 and ²³/₈ 1944, the former was found with a broken wing). The nematodes, which are long and slender, were found in the capsule of the adenohipophysis and partly stick in the glandular epithelium proper. They have caused only a slight reaction of the surrounding tissue. In addition, there are numerous large nematode eggs in the hypophyseal capsule, in the pars tuberalis, and in the connective tissue around the neurohypophysis. The eggs are surrounded by reaction tissue containing numerous polynuclear cells. Judging from the appearance of the parasites they are Filariids, but it was impossible to state their systematic position more exactly.

CHAPTER VIII

The Embryonic Development of the Neurohypophysis

Introduction and Literature

The description of the ontogenesis of the avian neurohypophysis is used here as an introduction to the chapters dealing with the neurohypophysis. This proved necessary, for the structure of this part in the adult can be understood only if its ontogenetic history is considered.

Some investigators of the embryology of the avian pituitary have also devoted a few lines to the neurohypophysis. The authors of the 19th century mainly discuss the interesting fact that the neurohypophysis is derived from the floor of the diencephalon whereas the adenohypophysis comes from the ectoderm of the stomodaeum. After 1900 the reports are more detailed. Tilney (1912), Haller v. Hallerstein (1934, p. 300), Griffiths (1938), Atwell (1939) and especially Rahn (1939) have described how the saccus infundibuli in chick embryos gives rise to hollow, finger-like buds which later form the neural lobe. As will be shown below this mode of differentiation plays a considerable rôle in the ontogenesis of the neurohypophysis in *Gallus*. However, the later embryonic stages are characterized by a second kind of differentiation, called diffuse emigration of cells in the present paper. This process plays a dominant rôle in many species of birds. Shanklin (1944) studied the development of the pituicytes in the chick embryo after staining by a modified Horteaga silver carbonate method, cp. p. 185.

Pfeiffer (1926) describes two different neural lobes in the chick embryo, which are believed to succeed each other ontogenetically (cf. p. 182). Hillemann (1943) made experimental lesions in the hypothalamic region of young chick embryos and observed the disturbances caused. His experiments tend to show that the presence of an intact neurohypophysis is necessary for the development of Rathke's pouch and for its early differentiation. The epithelial pituitary, on the other hand, influences the development of the neurohypophysis and seems to be necessary for its normal differentiation.

Apart from a few remarks on the neurohypophysis in duck and pigeon embryos (e. g. De Beer 1926, Lups 1929, Rost 1939, Painter 1942) showing that the organ arises by proliferation from the floor of the diencephalon I have found no modern descriptions of the embryonic neurohypophysis in other species.

The most important terms used in this chapter are explained in fig. 1 (p. 23). The finger-like median process which arises from the diencephalic wall near the top of Rathke's pouch in young embryos is called *saccus infundibuli*. It later gives rise to the infundibular stem and the neural lobe and has sometimes been called infundibular process. The latter term had to be avoided, however, for it is also used as a synonym for the neural lobe (cf. table 3). The term *infundibulum* has been used very differently by different authors. This term I use to designate the same as in the adult, viz. the eminentia mediana and the infundibular stem together. The embryological results indicate, however, that the structures covered by this term do not form a natural morphological unit. As the eminentia mediana is defined mainly on a functional (histological) basis this is true also for the infundibulum, but for birds in general it is tolerably well defined also from a morphological point of view. It comprises the diencephalic floor from the chiasma to and including the infundibular stem. The lumen contained in the infundibulum and the neural lobe is called *recessus infundibuli*.

Gallus gallus

Embryos 0—4 days. Though Rathke's pouch is well developed in the older of these embryos nothing is seen of a *saccus infundibuli*. In the 3-day embryo the diencephalon is distinctly delimited in a median section by the torus transversus rostrally and the tuberculum posterius caudally (cf. Kühlenbeck 1936). The chiasma is indicated by a slight thickening of the brain wall and by a delicate layer of fibres lying near the external surface. The chiasma is not, however, delimited posteriorly from the parts which will form the neurohypophysis. As the *saccus infundibuli* is still absent it is also impossible to state the posterior limit of the prospective neurohypophysis. The wall which will form the neurohypophysis is moderately thick and the nuclei of the cells within it are present from the inner to the outer surface.

In the 4-day embryos the chiasma opticum is much better developed, and the rostral limit of the prospective neurohypophysis is thus distinct. The true chiasma is still small, but the primitive supraoptic decussation is mighty (cf. Kühlenbeck 1937), and behind the latter we find a distinct aggregate of cells corresponding to the "nucleus a" of Rendahl (1924). The further development shows that the caudal end of this nucleus is included in the rostral part of the eminentia, but as stated by Rendahl (1924) the nucleus soon becomes diffuse and indistinct. The caudal limit of the neurohypophysis is still uncertain and its wall has the same appearance as in the 3-day embryo as regards the distribution of nuclei (fig. 94).

In the 3- and 4-day embryos the surface of the brain is covered by a delicate membrane, the prospective intima piaie (*membrana accessoria*, Held 1909). In azan preparations it appears as a fine blue line and by Gömöry's technique it can be shown that it consists of a delicate and dense net of argyrophilic fibres.

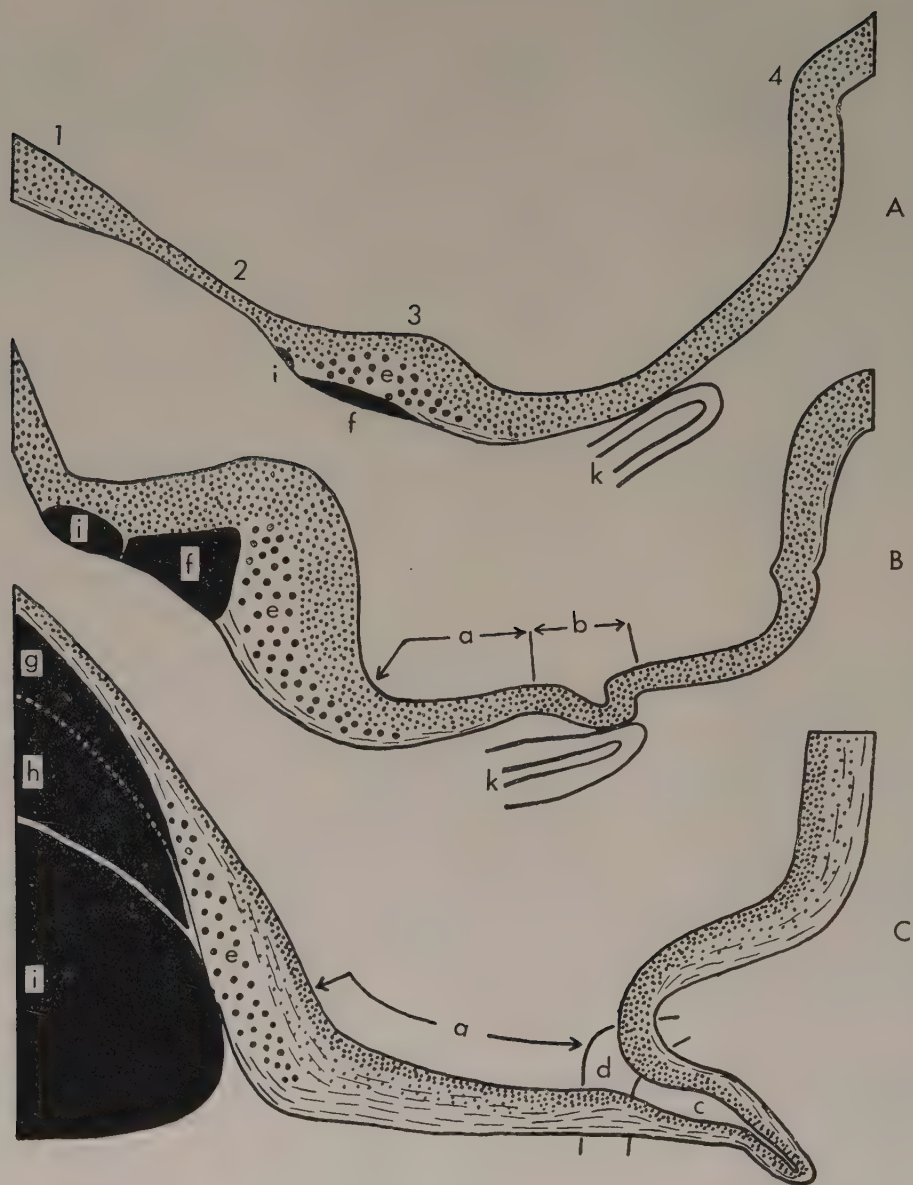


Fig. 94. The differentiation of the neurohypophysis in *Gallus*. The dots mark the distribution of the cell nuclei, the short streaks mark the nerve fibres. Median sections of embryos aged 4 days (A), 5 days (B), and 11 days (C).

1 = torus transversus, 2 = recessus praeropticus, 3 = chiasma thickening, 4 = tuberculum posterius.

a = eminentia mediana, b = saccus infundibuli, which later gives rise to the neural lobe (c) and the infundibular stem (d), e = "nucleus a" of Rendahl, f = primitive supraoptic decussation, which later gives rise to the dorsal (g) and ventral (h) supraoptic decussations, i = chiasma opticum, k = Rathke's pouch. All figures 85 $\times$.

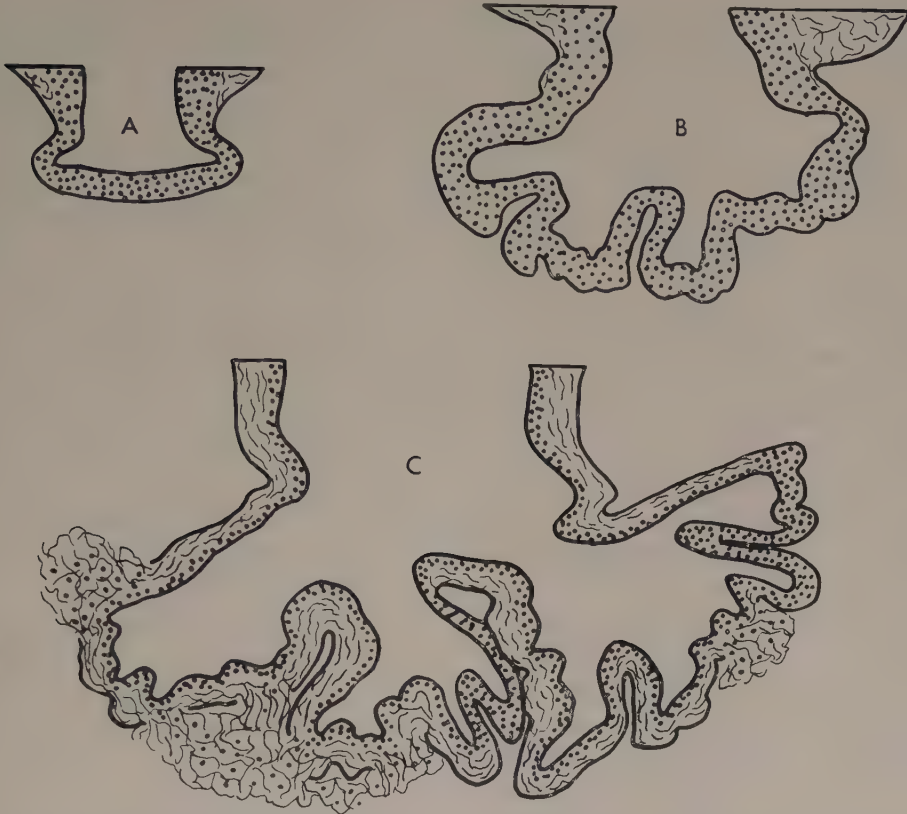


Fig. 95. The development of the neural lobe in *Gallus*. The planes of the figures are horizontal and parallel to the longitudinal axis of the organ. The dots mark the distribution of nuclei, the short lines mark nerve fibres.

A. Embryo, 6 days. The primary branches are present as lateral pouches. B. Embryo, 9 days. The hollow buds have appeared. No nerve fibres in the prospective neural lobe. C. Newly-hatched chick. Diffuse emigration distinct from some surfaces, a fibre layer extends over most parts of the neural lobe.

B and C are camera lucida drawings of horizontal sections, A is a graphic reconstruction from a series of cross sections. A and B 130 $\times$, C 65 $\times$.

Embryos 4.5—7 days. A distinct saccus infundibuli is present in both my 4.5-days embryos and must thus arise in the first half of the 5th day of incubation. As can be seen in older embryos this structure will give rise to the neural lobe and infundibular stem, and it is thus possible to delimit the prospective neurohypophysis exactly in a median section of these embryos (fig. 94 B). The saccus infundibuli is still a shallow fold near the top of Rathke's pouch in the 4.5- and 5-day embryos (fig. 55, 56 A). In the 6- and 7-day embryos it is deeper and broad from side to side, especially near the top where a pair of lateral diverticula are being formed (fig. 95 A, 56 B, 57). These first diverticula from the saccus are

constant in all the species which I have examined and give the saccus the shape of a broad T or Y with the base attached to the brain. They will be called *primary branches*. They are the first step of the proliferation process which later gives rise to the neural lobe from the top of the saccus. The base of the saccus remains narrower and will later form the infundibular stem.

In the 7-day embryo the primary branches have grown out latero-caudally and there are even indications of additional buds. Thus, the left branch is forked at the point and another process has been formed from the median wall between the primary branches. All these parts contain lumina which are continuous with the recessus infundibuli.

Important changes can be observed also in the prospective eminentia mediana, i. e. the region between the saccus and the chiasma in a median section. The rostral, post-chiasmatic part is distinctly thickened in the 4.5- and 5-day embryos and contains the caudal parts of the "nucleus a" of Rendahl. An invasion of nerve fibres has also begun here and is visible in azan preparations as a narrow, fibrous zone along the external surface (fig. 96 B). The nuclei of the cells have become restricted to the inner part, and only the slender protoplasmatic processes traverse the fibrous zone and attach to the external membrane with broad "vascular feet". The fibrous layer of the eminentia is narrow in the median parts but becomes broader laterally. It has not reached the region around the saccus infundibuli in embryos of this age and increases in thickness towards the chiasma.

Bodian impregnation reveals nerve fibres in this layer among the processes of the ependymal cells. All fibres which can be seen run parallel to the surface and come from rostral and lateral directions.

Embryos 8—10 days. The saccus infundibuli in these embryos is distinctly differentiated into an infundibular stem, which is the basal part, and the neural lobe. The primary branches do not become large in *Gallus* and are less distinct in embryos more than 8 days old because of the appearance of numerous hollow buds. The development of the latter, which are called buds here in order to avoid confusion with the primary branches, is often asymmetrical and irregular. They arise both from the primary branches and from the wall between them. In a few embryos of this and later stages there is even a median bud from the posterior ventral surface of the median eminence, without relation to the saccus infundibuli. It corresponds to the "secondary diverticulum", drawn by De Beer (1926, fig. 37). Such diverticula are fairly rare and highly variable. They will be discussed later because they were used to support a peculiar hypothesis by Pfeiffer.

The development of the buds is widely different from the type of proliferation which occurs later and will therefore be described in detail. No nerve fibres are as yet visible in the proliferating walls of the prospective neural lobe, and the nuclei of the cells are present from the internal surface nearly to the external membrane. When a bud is formed the wall just bulges out and the new bud thus immediately acquires a lumen. The cell nuclei, and of course also their plasmatic cell bodies, thus enter the new bud at once. In many cases the nuclei even seem

to be pressed against the surface at the top of the growing bud. The external reticular membrane (intima pia) remains continuous during this process and does not show any openings (fig. 95 B).

The stem is distinct in these embryos and the eminentia mediana has increased in thickness. The superficial fibrous layer of the eminentia becomes thicker and reaches the base of the infundibular stem, in the oldest of these embryos even the proximal parts of the neural lobe. Near the chiasma the fibre layer is no longer free from nuclei, but has been invaded by a glia nuclei from the internal nuclear layer of the wall.

In the 7-day embryo the coarse nerve fibres of the eminentia have receded from a narrow space along the external reticular membrane, and this space becomes more distinct in older embryos. This new zone is called "glandular layer" because of its supposed glandular function in the adult. The only visible cellular elements in this zone are the numerous processes of the ependymal and glia cells which traverse it and attach to the outer surface of the wall (cf. fig. 96). This zone often stains more reddish in azan than the fibre layer inside, which stains more bluish. The increased stainability with azocarmine does not depend only on the absence of nerve fibres, for the ependymal processes do not show this staining reaction in the inner parts of the wall. The reddish colour of the glandular zone is very distinct already in an 8-day embryo and seems to be caused by a fine granular substance of unknown significance.

Embryos 11 days—adult. The further growth of the neural lobe is characterized by excessive development of the hollow buds. The primary branches are little distinct, become dislocated rostrally and form the lateral extremities of the organ (fig. 95). The most interesting process during the second half of the incubation period is, however, the "diffuse emigration" of nervous matter into the surrounding mesenchyma. The diffuse emigration begins on the 11th day of incubation and is apparently caused by the invasion of nerve fibres into the neural lobe. By this time the fibre sheath of the median eminence has extended over the infundibular stem and also to some buds of the neural lobe, which are thus covered by a fibrous layer of the same kinds as is the eminentia. A glandular zone has not appeared in these parts, however. When diffuse emigration of cells takes place the intima pia opens in some well defined places and fibrous matter together with stainable colloid from the nervous wall spreads into the interstitia of the surrounding mesenchyma (fig. 95 C). This material which emigrates first through the opening in the intima comes from the superficial fibrous layer of the wall. Later also some ependymal cells loose contact with the internal nuclear layer in the wall and enter the colloidal clouds in meshes of the mesenchyma. Whereas the ependymal cells in the original wall usually have long processes which reach the outer surface these migrating glioblasts become irregular and have a more contracted cell body. The new parts of tissue formed in the mesenchyma by this diffuse emigration of nervous elements is called *secondary lobes*.

In azan preparations where the intima pia is blue or in Gömöry preparations

where it is black, it is distinctly seen that the membrane really opens and lets out the content of the nervous wall into the mesenchymatic net. Thus there is a complete mixing of nervous and mesenchymatic elements, and blood vessels in the mesenchyma are regularly included in the secondary lobes. The diffuse emigration of cells takes place from several points at the same time, both from the walls of the primary branches and from the hollow buds. When it begins in the 11-day embryo the neural lobe consists of the two primary branches and numerous hollow buds. Usually the secondary tissue is formed to a very restricted extent in *Gallus* and only occupies some spaces between the hollow buds in the hatched chick and the adult (fig. 95). It should be remarked already here that the variations from one specimen to the other are very great. Some adult glands are formed almost exclusively by hollow buds, and the secondary tissue forms a relatively inconsiderable part. Other glands are made up almost exclusively of secondary tissue, which makes the gland look more compact with a very restricted lumen. These variations were observed in the adult fowl by Obussier (1948).

The changes occurring in the median eminence from the 11th day are not very great. The three layers characteristic of the adult are present from the 7th day: an external glandular layer, an internal ependymal layer with the nuclei of the ependymal cells, and an intermediate fibrous layer containing the coarse nerve fibres (fig. 96). In the eminence the ependymal cells originally reached from the inner to the outer surface of the wall. When the superficial fibre layer appears the main bodies of the cells and their nuclei are restricted to an inner "ependymal" layer, and long slender processes retain the contact with the external surface. These processes later become branched, especially in the zone which is called glandular layer, so that they reach the external surface with numerous "vascular feet". It seems probable from observations in azan preparations that this branching is preceded by a thickening of the peripheral end of the process, and that the thickened part later is divided into separate strings (fig. 96 C). The glioblasts which leave the ependymal layer and settle in the fibre bundles of the tractus hypophyseus also send arborized processes towards the external surface and sometimes also a single string into the ependymal layer.

The walls of the infundibular stem, primary branches, and the hollow buds of the saccus infundibuli are developed in the same way as the eminentia, but the walls are thinner and there are rarely any free cells among the tractus fibres. The glandular layer is narrower and occurs later than in the eminence. The secondary tissue, formed by emigration of material from the neural wall, looks quite disordered when it first appears in the mesenchyma. The older secondary lobes soon begin to reorganize, however, and acquire a structure somewhat resembling that of the primitive walls. The glioblasts usually have a simple spindle-shaped or rounded cell body when they arrive through the opening in the intima. Later, when a considerable mass of secondary tissue has been formed, they begin to send processes towards the capillaries and assume a more or less star-shaped body. The processes of these glia cells are fairly few, usually 1—4. Their cell bodies become

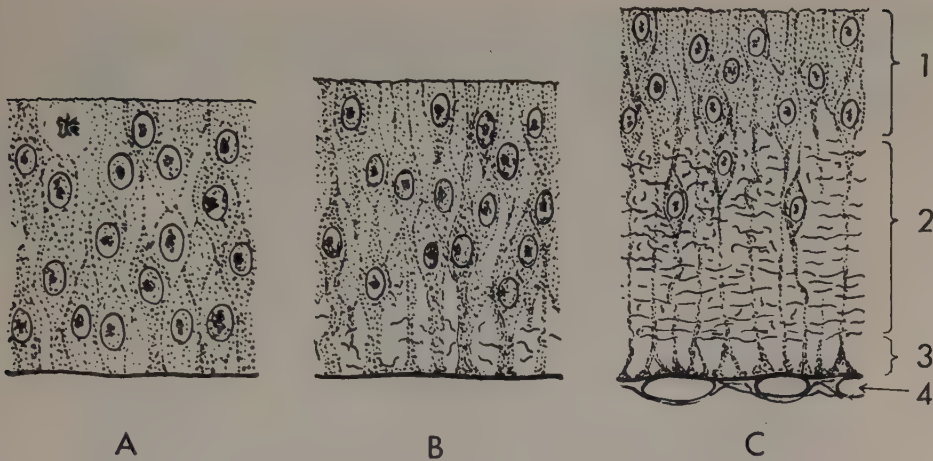


Fig. 96. The histogenesis of the eminentia mediana in *Gallus*. Median sections of embryos aged 4 days (A), 5 days (B), and 11 days (C). The layer of nerve fibres has appeared in B, and in C the three characteristic parts of the eminentia are distinct.

1 = ependymal layer, 2 = fibre layer, 3 = glandular layer, 4 = superficial capillary net outside the black line which marks the intima piae.

Slightly schematic camera lucida drawings, from azan and Bodian preparations. 600 $\times$.

concentrated at some distance from the vessels together with the coarse nerve fibres, and the capillaries thus become surrounded by spaces free from coarse nerve fibres and traversed by the vascular processes of the glia cells. These perivascular spaces can be compared with the glandular layer of the median eminence and of the primitive walls in the neural lobe, which are covered by a dense capillary net. In both cases we have a zone next to the vessels free from coarse nerve fibres and nuclei and traversed by vascular processes of the ependymal or glia cells.

Larus ridibundus

Embryos, CR 3—12.5 mm: The saccus infundibuli is still barely indicated in the 7.5 mm embryos as a shallow pouch from the diencephalic wall just above the top of Rathke's pouch. The chiasma is also indistinctly delimited from the eminentia, so the limits of the neurohypophysis are rather vague. A thin superficial layer with fibres has begun to invade the eminentia, but does not reach far caudally. In the older embryos the saccus infundibuli is deeper, and in the 12.5 mm embryo it is very broad from side to side. In these older embryos the superficial fibrous layer is still absent from the region around the saccus.

Embryos 13.6—18 mm: In the 13.6 mm embryo the saccus infundibuli is distinctly bifid at the end and the two primary branches thus formed extend latero-caudally (cf. fig. 97 A and 98). They show a tendency to give rise to buds, for they are both slightly forked at the end. This condition is further developed in the older embryos (CR 18 mm), in which the primary branches are very long and sometimes

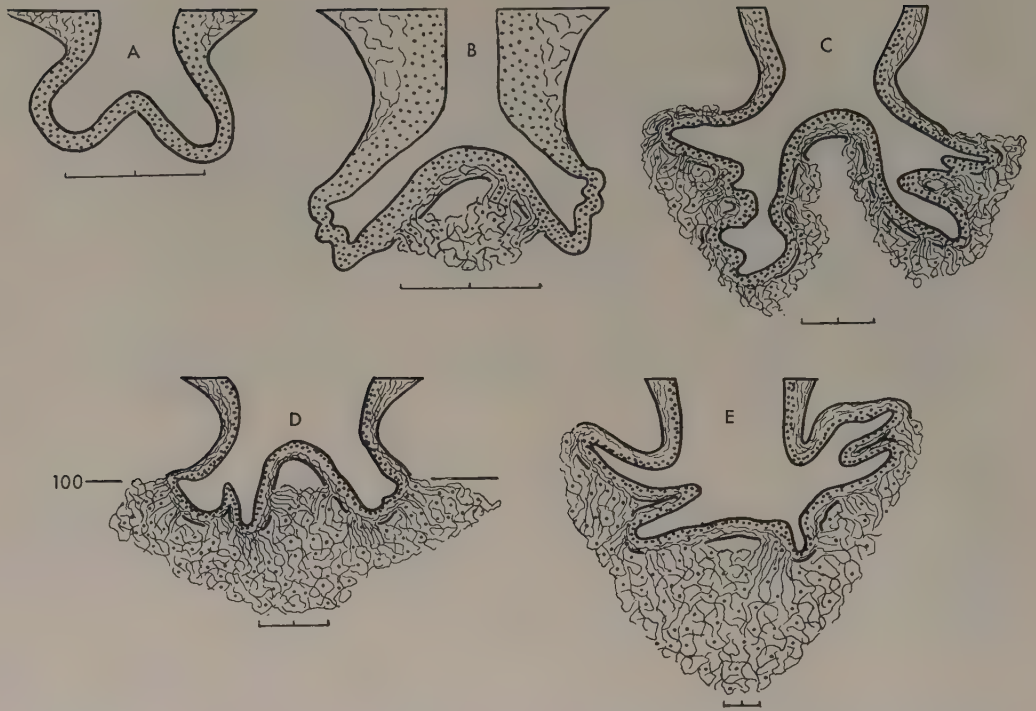


Fig. 97. The development of the neural lobe in *Larus ridibundus*. The horizontal plane of the figures is parallel with the longitudinal axis of the organ. The dots mark cell nuclei, the lines mark nerve fibres. A = CR 18 mm, B = CR 29, TL 43 mm, C = TL 78 mm, D = TL 94 mm, E = ♀ ad.

Fig. B is a camera lucida drawing of the same embryo as fig. 99, the other figures are graphic reconstructions from section series. 100 in fig. D marks the plane of fig. 100. The line under each figure represents 0.2 mm in the same scale as the figure.

end in two or three small buds. The buds contain a lumen, and the intima on the surface is intact, the proliferation is therefore still of the primitive kind described as "budding" in the chick embryos. The fibrous layer of the eminentia is well developed in all these embryos and extends to the surface of the infundibular stem, but has not yet reached the neural lobe. The eminentia mediana shows the typical appearance with the ependymal cell bodies and nuclei near the inner wall, the fibres of the tractus hypophyseus near the outer wall, and the ependymal processes which penetrate the fibre bundles and attach to the surface by means of thickened "vascular feet".

Embryos 19.6—32 mm: The eminentia and the stem are not much different from the preceding stage. The fibrous layer has grown thicker and has invaded the stem and the basal parts of the primary branches. Some cells in the ependymal layer of the eminentia have left this zone and entered the fibrous layer in the 19.6 mm embryo and this invasion of glia cells into the tractus bundles continues in older embryos.

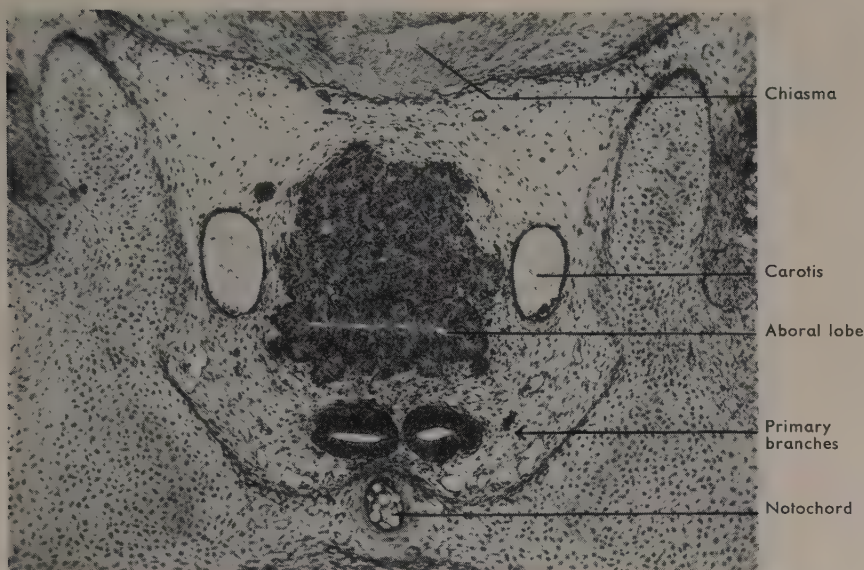


Fig. 98. *Larus ridibundus*, embryo CR 18 mm. Horizontal section. The primary branches are well developed and diffuse emigration has not started. Rostral end upwards in the figure. — Susa, paraffin 6μ , azan, $80\times$.



Fig. 99. *Larus ridibundus*, embryo CR 29, TL 43 mm. Horizontal section through the longitudinal axis of the saccus infundibuli, showing the large primary branches and beginning of diffuse emigration. — Susa, paraffin 6μ , azan, $90\times$.

In addition to the two primary branches in the neural lobe, each with 2—3 buds, some embryos also have a median bud from the top of the original saccus infundibuli between the two primary branches. In the 19.6 mm embryo there is still no diffuse emigration of nervous elements into the mesenchyma, but the delicate fibres of the intima have become loosened and sparse in some patches near the ends of the two primary branches, so an emigration seems to be prepared here. The same is true also for the 20 mm embryo, and in the Gömöry-impregnated slides of the 25 mm embryo a diffuse emigration of nervous matter could be stated from several points near the ends of the two primary branches. In the still older embryos this process has already advanced far. The diffuse emigration is usually most intensive from the ramified ends of the primary branches and from their median surfaces, but may sometimes occur also from the unpaired median process if it is present or from the original wall at the top of the saccus between the branches (fig. 97 B, 99). As in *Gallus* the matter which first emigrates is a colloid containing a fibrous structure, and the glioblasts do not appear outside the wall until later. In the 30—32 mm embryos there are considerable bodies of secondary tissue round each of the primary branches, and a fusion has usually taken place in the mid-line so the neural lobe has an unpaired appearance. In some cases, however, there are two separate bodies both attached to the infundibular stem by a stalk consisting of the basal part of the primary branch (fig. 97 C and D, 100).

Embryo 35 mm—adult. In the later part of the ontogenesis in *Larus ridibundus* the diffuse emigration of nervous matter continues from the medial walls and the ends of the primary branches, whereas the antero-lateral walls of the branches often retain their primitive, eminentia-like structure. The adult pituitary distinctly reflects this development: There is an unpaired stem which gives rise to two more or less distinct branches near the base of the neural lobe (cf. fig. 97 D and E). These branches then continue laterally along the sides of the main neural lobe. They give rise to 2—3 smaller buds each of which sometimes pass independently into the body of secondary tissue. The stem and also the non-proliferated walls of the primary branches and buds show the primitive, eminentia-like structure, but the greater part of the neural lobe, especially the caudal and central parts, are formed by secondary tissue. Even in embryos as large as 78 mm (TL) the neural lobe may be distinctly paired, with two bodies of secondary tissue, one around the top of each primary branch, and separated by non-invaded mesenchyma. It is highly probable that also adult birds sometimes have a paired neural lobe of this appearance, but I have none in my collection. The paired development of the neural lobe explains the fact why it is almost impossible to get a median section in which the lumen of the neurohypophysis is continuous.

The reorganization of the secondary tissue begins in the first formed secondary lobes of embryos measuring 35 mm CR. The invaded cells send out vascular processes and are transformed in the same way as in *Gallus*. The changes occurring in the eminentia and the stem are not very different from those in *Gallus*.

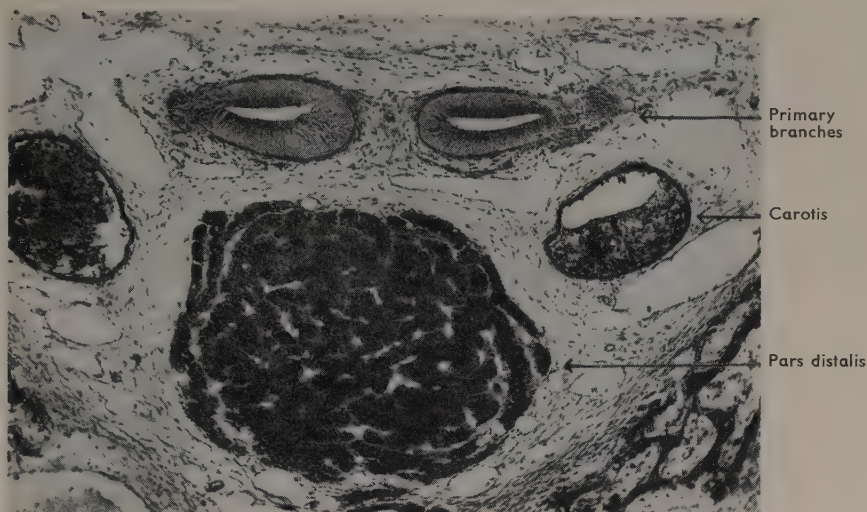


Fig. 100. *Larus ridibundus*, embryo TL 94 mm. Transversal section through the posterior end of the pituitary (plane 100 in fig. 97 D). The primary branches are still separate at this level and diffuse emigration takes place from their lateral surfaces. — Susa, paraffin 6 μ , azan. 85 $\times$.

Riparia riparia

Embryos CR 3.5—10.5 mm. The 5 mm embryo has a slight outpocketing from the diencephalic wall as a first indication of a saccus infundibuli. The chiasma is not yet distinctly defined and the prospective neurohypophysis is only approximately recognizable. In the larger embryos the saccus infundibuli is deeper, and in the 9.7 and 10.5 mm embryos the basal parts form a distinct stalk whereas the top of the saccus is dilated laterally and has developed a pair of primary branches, which are only shallow pouches and never reach any considerable length in this species (fig. 101). The prospective neural lobe and the infundibular stem can thus be approximately recognized at this stage. In all embryos more than 5 mm CR the eminentia is distinct from the well developed chiasma. The superficial fibre layer in the eminentia is still poorly developed in the 8.5 mm embryos but is distinct in older specimens. In the 9—10.5 mm embryos the fibre layer has even extended to the infundibular stem and the neural lobe, but there is some variation in the thickness and extension of this zone.

10.5 mm—adult. In some 10.5 mm embryos a diffuse emigration of nervous matter has started from the dilated top of the saccus infundibuli, and this process continues until the adult gland has been formed. The neurohypophysis in the adult sand-martin (fig. 102) is thus exclusively formed by secondary tissue. The primary branches are only indicated as shallow pouches of the lumen and no hollow buds are developed as in the two preceding species. The emigration takes place from



Fig. 101. *Riparia riparia*, embryo CR 10.5 mm. Horizontal section. The saccus infundibuli is dilated into a pair of primary branches which are poorly developed in this species. — Bouin, paraffin $10\ \mu$, azan. $80\times$.

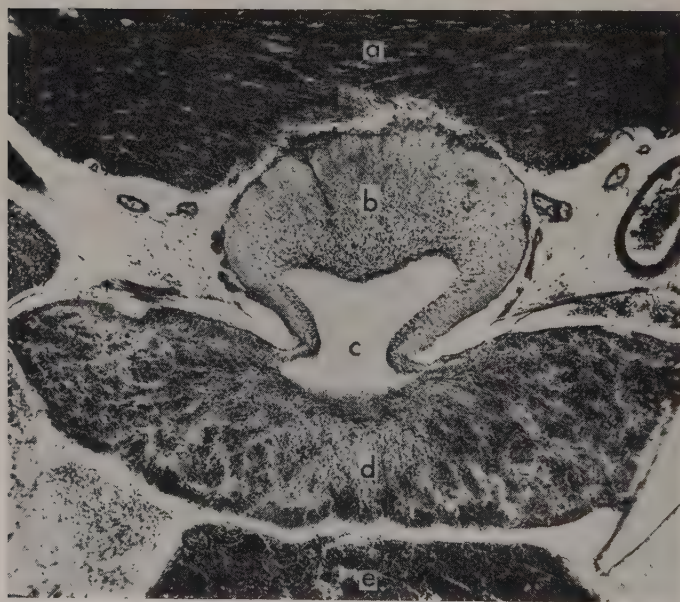


Fig. 102. *Riparia riparia*, ♀ ad. Horizontal section through the chiasma (a), the eminentia mediana (b), the infundibular stem (c), the neural lobe (d), and the pars distalis (e). — Bouin, paraffin $10\ \mu$, azan. $70\times$.

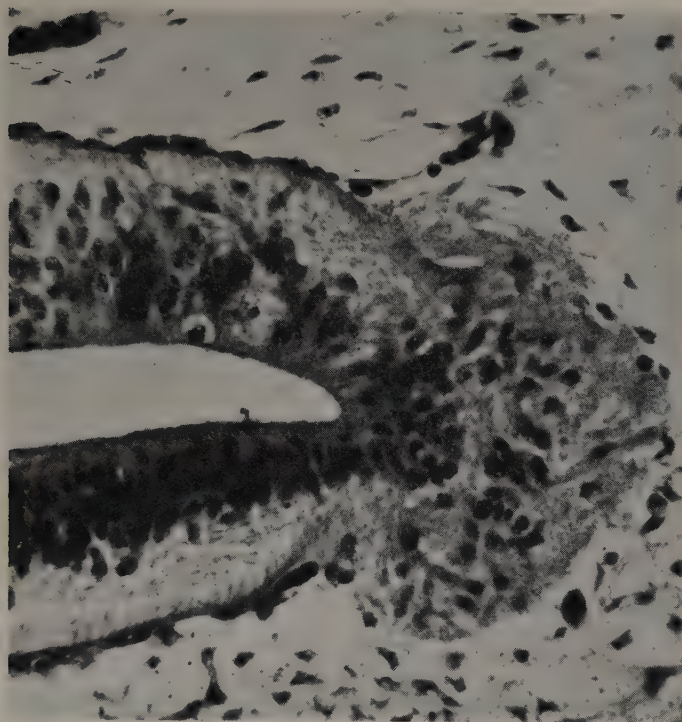


Fig. 103. *Fulica atra*, embryo CR 29 mm. Median section. Diffuse emigration from the posterior surface of the saccus. — Bouin, paraffin 8 μ , azan, 500 $\times$.

the entire posterior surface of the neural lobe and from the evaginated lateral parts. There is certainly a relation between the appearance of the fibre layer on the surface of the neural lobe and the diffuse emigration of cells in all the three species. Also in *Riparia* it could be stated that diffuse emigration never starts before the fibre layer appears in the prospective neural lobe. The emigration first gives rise to a flattened broad body of secondary tissue, and growth in a dorso-ventral direction does not commence until the embryo is nearly full-term. The secondary tissue is reorganized in relation to vessels and connective tissue as in *Gallus* and *Larus*. As will be clear from the description above the adult neural lobe in *Riparia* is compact and the lumen of the stem does not reach far into it.

The development of the eminentia mediana and the infundibular stem does not present any essential features not described for *Gallus* and *Larus*.

Scattered Observations in Other Species

Turdus pilaris. One clutch of embryos is available, but all are of different size (CR 15, 18, 19, 21 and 23 mm). This indicates that incubation had begun as soon as the first egg was laid. In the 15 mm embryo the saccus infundibuli is a distinct

and deep pouch, extended into a pair of primary branches at the end. There is a fibre layer on the eminentia and on the basal parts of the saccus. Similar conditions were observed in the 18 mm embryo. In the 19 mm embryo the primary branches are divided into two hollow buds at the end, and the fibre layer of the eminentia has extended all over the neural lobe. Diffuse emigration of neural tissue has begun along the entire posterior surface of the neural lobe. In the older embryos this process has resulted in distinct secondary lobes. In the adult practically all parts of the neural lobe are formed by secondary tissue (fig. 111). The lumen of the stem enters the neural lobe and continues as two symmetrical channels (primary branches) which reach the lateral extremities of the gland. The gland is, in fact, of the same type as in *Riparia*, but the lumina are better preserved, and the primary branches much more distinct.

Fulica atra L. Two embryos, measuring 28 and 29 mm CR, are essentially alike. The saccus infundibuli is differentiated into a stem and a neural lobe, which has given rise to a pair of long primary branches directed latero-caudally. Both branches are slightly bifid at the top and have a distinct external fibre layer in the wall. Extensive cell emigration occurs from the medial surfaces of the branches and also from the wall between their bases (fig. 103). In another embryo, measuring 27 mm CR, the same could be seen, but in addition there is also a median bud from the wall between the two branches. The development in this species seems to be very much like that in *Larus* as far as the neurohypophysis is concerned.

Conclusions and Discussion

The earliest stages. The areas of origin of the different parts of the neurohypophysis can be recognized in the embryos as soon as the saccus infundibuli has been formed (*Gallus* 4.0—4.5 d., *Larus* CR 7.5 mm, and *Riparia* CR 5.0 mm). The entire area between the chiasma thickening and the base of the saccus gives rise to the eminentia mediana. The base of the saccus infundibuli forms the infundibular stem and its top proliferates and forms the neural lobe. This early differentiation of the prospective neurohypophysis into two morphologically distinct structures, the eminentia and the saccus, justifies the terminology used here. The infundibular stem, which is derived from the saccus, and the eminentia should thus be kept apart as far as possible but in adult birds this is often very difficult in practise (cf. pp. 188, 237).

As evidenced by all five species described above the neural lobe is originally paired in birds. As soon as it appears it gives rise to a pair of hollow branches which later proliferate in different ways and may remain separate for a considerable part of the incubation period. The paired origin of the neural lobe is often distinct also in adult birds. This fact is interesting if compared with the conditions in *Petromyzon marinus*, described by Tilney (1937, 1938 fig. 20). In this animal the "infundibular process" which is surrounded by a typical intermedia, is distinctly paired. Tilney's theories about the relationships between saccus vasculosus and

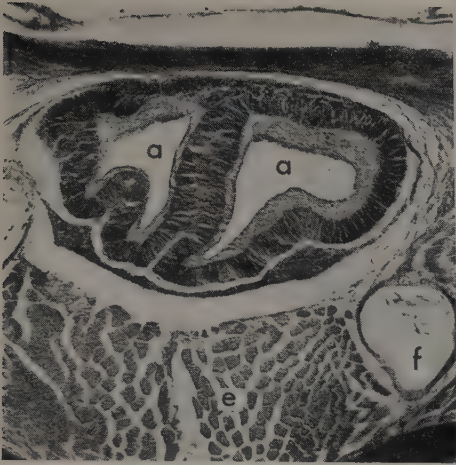


Fig. 104.

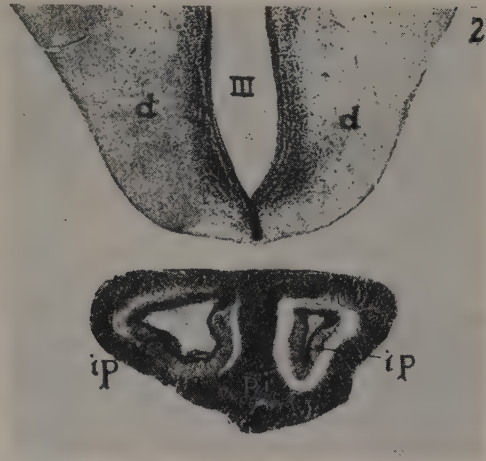


Fig. 105.

Fig. 104. *Tarentola delalandei*, ♀. Transversal section through the posterior end of the pituitary, showing the paired neural lobe. The recessus infundibuli (a) is surrounded by a thin nervous wall (light), and the whole neural lobe is surrounded by a mighty intermedia (black). The second dark wall below is the pars distalis. e = musculus retractor oculi, f = carotis. — Bouin, paraffin 15 μ , acid fuchsin — phosphomolybdic acid — light green. 80 $\times$.

Fig. 105. *Petromyzon marinus*. Cross section of brain of adult. From Tilney (1938, fig. 20). d = diencephalon, ip = "infundibular process", pi = "pars infundibularis" (intermedia). 44 $\times$.

neural lobe can not be discussed here, but it seems probable that the pair of hollow diverticula from the brain in *Petromyzon* really correspond to the neural lobe in amniotes, particularly because they are invested by a thin layer of the adenohypophysis (cf. p. 244 ff.). A paired condition of the neurohypophysis can be traced also in many reptiles, as I have been able to state in my own material. The two diverticula are especially large in *Tarentola delalandei*, which is shown in fig. 104, and in this species the double sac-like neural lobe is invested by a thin layer of intermedia just as in *Petromyzon*. Cross sections of the neural lobe in these two species therefore look almost completely alike (fig. 104 and 105). Maybe the neural lobe is a paired organ originally and the paired development in birds thus has a phylogenetic explanation. Also in *Sphenodon* there is a paired neural lobe (Wyeth and Row 1923).

The formation of hollow buds. The proliferation of the neural lobe begins with the formation of hollow buds from the primary branches of the saccus infundibuli and in some cases also from the median surface between them. In many specimens of *Gallus* the buds become very numerous and constitute the main part of the adult neural lobe, whereas the diffuse emigration of cells gives rise to fairly small portions of tissue. In *Larus*, and probably also in *Fulica*, each primary branch gives rise to 2—3 such buds, and the main part of the adult organ is secondary tissue, and in *Turdus* the buds seem to be poorly developed. In *Riparia*,

finally, no hollow buds at all are formed, and the adult neural lobe is built up exclusively by diffuse emigration of nervous elements.

The buds are formed as hollow, finger-like diverticula from the original wall. The structure of the wall is thereby not disordered, but retains the typical appearance with the regular layer of ependymal cells. The reticular membrane on the surface (intima piaie) remains intact and separates the nervous elements inside from the mesenchyma outside.

The formation of hollow buds appears to be a much simpler process than the diffuse emigration and is perhaps also primitive from a phyletic point of view among the amniotes. This is supported by the fact that the neurohypophysis in some reptiles, especially *Sphenodon*, probably grows in a way similar to the process called "budding" above. According to Wyeth and Row (1923) the neural lobe of *Sphenodon* is a slightly wrinkled and paired sac, and its wall contains an external fibre layer and an internal ependyma just as the walls of the typical "buds" in birds. It may be mentioned also that such a primitive neural lobe is present also in some lacertilians and chelonians, a fact which I have observed in my own material.

As mentioned above on p. 170 it sometimes occurs in the chick that a bud is formed from the ventral surface of the median eminence, far from the saccus infundibuli. This bud was observed also by Pfeiffer (1926), who interpreted it in a way which is open to criticism. He observed the formation of the saccus infundibuli (called infundibulum in P.'s paper) after 5 days of incubation, but then he found a "second infundibulum" growing out from the floor of the diencephalon, rostrally of the original one. This "second infundibulum" is said to establish a contact with the adenohypophysis just as the first one, and to migrate posteriorly. On the 13th and 14th day of incubation the first "infundibulum" is said to degenerate and to be replaced by the second. Pfeiffer is of the opinion that the posterior and anterior "infundibulum" correspond to Seessel's and Rathke's pouches resp., and that the disappearance of the posterior infundibulum is correlated with the degeneration of Seessel's pouch. This view is very strange, and I have found no support for it. The median bud from the eminentia, which is certainly the structure interpreted as a second "infundibulum" is present in less than half of my chick embryos more than 7 days old. It is extremely variable and does not show any tendency to exceptional growth, nor does the saccus infundibuli show any signs of progressive degeneration. The bud from the eminentia is probably a result of the general tendency in this region to form buds, and the only curious thing is its exceptional situation outside the saccus. Certainly it has no comparative morphological significance. In later embryos the buds from the eminence give rise to secondary tissue, but the amount is fairly small and I have rarely seen any remnants of this exceptional structure in adult birds.

The growth of the tractus hypophyseus. Formation of hollow buds has not been seen from surfaces which have been invaded by nerve fibres, and, on the contrary, diffuse emigration of nervous matter was never seen from non-invaded walls. It

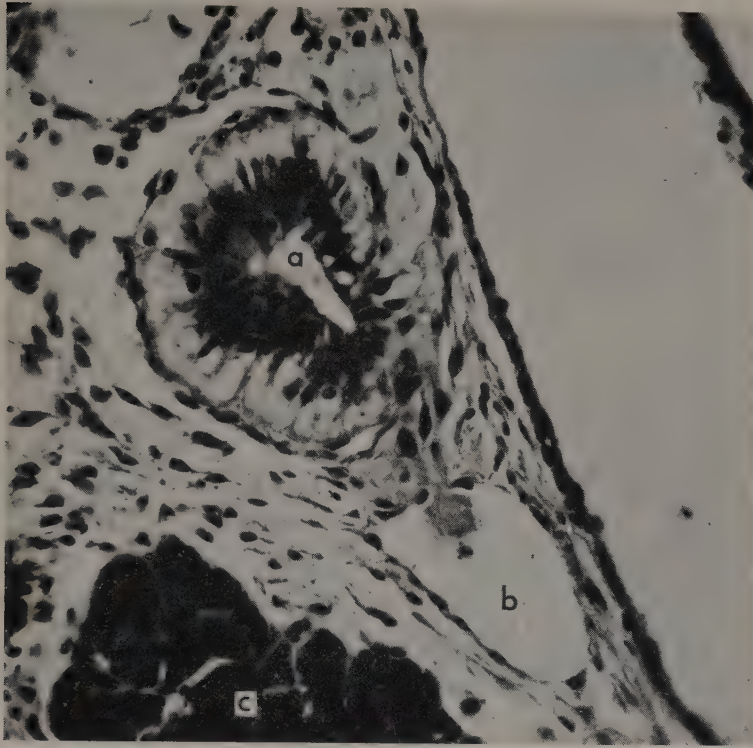


Fig. 106. *Riparia riparia*, embryo CR 19 mm. Paramedian section through one primary branch (a) from which diffuse emigration takes place. One of the tongues of emigrating tissue has entered a blood vessel (b) in which the colloid spreads like a cloud. c = pars distalis. — Bouin, paraffin 8 μ , haematoxylin of Ehrlich and eosin. 430 $\times$.

thus seems probable that the change in the mode of proliferation is directly caused by the appearance of the nerve fibres in the neural lobe.

Details of the "diffuse emigration of nervous matter". As mentioned above the nerve fibres seem to play a leading part in the diffuse emigration of nervous matter, and in fact this is corroborated by the details of the process. When the intima on the surface is opened and partly pushed aside, the mesenchyma is first invaded by a colloidal mass which contains delicate fibres, probably belonging to the tractus hypophyseus. The fibres are difficult to impregnate distinctly, but in a few Bodian preparations it could be seen that they are continuous with the fibres in the original wall of the bud. The colloid may be structureless in living tissue, but is precipitated in a granular state by the fixatives and stains readily with eosine. The colloid is fairly independent of the nerve fibres, but is probably secreted by them. It does not seem likely that all the colloid pours out from some source in the original wall, for the volume of the saccus is very small in relation to the emigrating colloid masses in *Riparia*. The fact that the colloid is independent

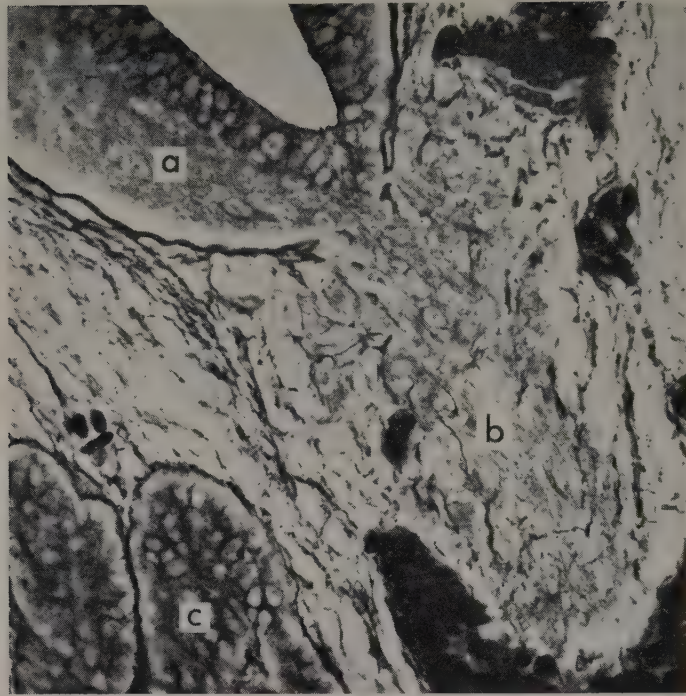


Fig. 107. *Larus ridibundus*, embryo CR 28 mm. The paramedian section shows the diffuse emigration of nervous matter into the meshes of the mesenchymatic fibres (black). a = saccus infundibuli, b = secondary lobe, c = pars distalis. — Susa, paraffin 8μ , impregnation of reticular fibres according to Gömöry, contraststaining with azocarmine. $480\times$.

of the nerve fibres is beautifully shown in some specimens of *Riparia*, in which the emigrating mass has penetrated the wall of a large sinusoid capillary in the mesenchyma. The colloid has then spread like a cloud into the lumen of the vessel, while the fibres have just reached the wall of it (fig. 106).

When also some ependymal cells in the original wall have emigrated and entered the colloid and fibres in the mesenchymal net as prospective pituicytes, the secondary lobe formed consists of the following elements: 1) nerve fibres, 2) intercellular colloid, 3) mesenchymatic cells and their processes (fig. 107), 4) pituicytes, invaded from the neural wall, and 5) small capillaries. This primary condition is later changed. The mesenchymatic fibres partly disappear, but a number of connective-tissue elements are always preserved in the secondary lobes, even in the adult. The nerve fibres and pituicyte bodies recede from the walls of the capillaries so that "perivascular spaces" are formed. The pituicytes emit processes which traverse these spaces and attach to the surface of the capillaries by vascular feet.

When parts of the neural lobe have been formed by hollow buds the histological appearance is distinctly different. The vessels and connective tissue elements are not present within the nervous tissue but only on the surface or in furrows on the

surface. The buds contain a more or less distinct lumen lined with ependymal cells. The zone along the superficial membrane which is covered by the capillary net attains the appearance of a glandular zone in the median eminence, and the nerve fibres are present in an intermediate layer. It is interesting that a substitute for this glandular zone may be found in the perivascular spaces of the secondary tissue. In both cases the nerve fibres and cell bodies are absent and the space is traversed by numerous vascular processes.

Addendum

Unfortunately I had not seen Shanklin's paper (1944) on the histogenesis of the pituicytes in the chick when my manuscript was sent to the printer, and I therefore add a short comment on it here. Shanklin used the Hortega silver carbonate method in addition to common haematoxylin preparations, whereas I have based my descriptions on azan, Gömöri and Bodian preparations. In spite of very different technique the interpretation of the development of the pituicytes is almost perfectly the same in Shanklin's paper and mine and this, of course, must be regarded as a strong support for the correctness of the conclusions.

Shanklin stated that the nuclei of the cells are present throughout the wall of the prospective neurohypophysis in the very young embryos. He also describes the appearance of an external zone, free from nuclei and traversed by the processes of the "spongioblasts" (= my-ependymal cells) in embryos about 9 days old. He also recognized the two kinds of proliferation in the neural lobe: formation of "primary lobules" (= my hollow buds) and of "secondary lobules" (= my secondary lobes). The reorganization of the apolar cells which invade the secondary lobes is described in almost perfect agreement with the statements in the present chapter. It is to be noted, however, that the chick is no favourable material for the study of the development of the secondary lobes.

Some points of disagreement should be commented on. Shanklin regards the external membrane on the prospective neurohypophysis as a *membrana limitans*, formed by the dilated ends of the processes of the spongioblasts. As stated above I also hold that such a membrane is present, but azan and Gömöri preparations show that this *membrana limitans* is covered by a reticular membrane of mesenchymatic origin. Azan and Gömöry preparations also show that the mesenchymatic net is actually infiltrated by the nervous tissue when the secondary lobes are formed, and that derivatives of the mesenchymatic net are common in the secondary lobes of the adult. This could evidently not be seen perfectly in Shanklin's preparations, but he states that the limiting membrane becomes indistinct when the secondary lobes are formed. Further Shanklin describes neuroblasts and nerve cells in the *eminentia mediana* and also in the stem and the neural lobe of embryos. He admits, however, that they may be inconstant in the neural lobe of the adult for he searched for them in vain in one specimen, and they are rare in the neural lobe also in chicks and embryos. I found nerve cells and neuroblasts in the *eminentia*, and they are especially common in the rostral parts ("nucelus a" of Rendahl), but I never found any in the neural lobe. The nerve fibres and the interstitial colloid are only occasionally mentioned by Shanklin.

On the other hand, Shanklin's skilful handling of the Hortega method allowed him to describe the development of the pituicytes more in detail than I have done, and the reader is referred to his paper for particulars. It is especially interesting that he, too, could see the excessive branching of the ependymal processes in the *eminentia mediana*, which gives rise to the dense wood of fine and parallel protoplasmic fibres in the glandular layer of the adult.

CHAPTER IX

The Structure of the Neurohypophysis in Adult Birds

Literature

The records in the literature concerning the structure of the neurohypophysis in adult birds are almost as fragmentary as those concerning its development. Müller (1871) found that the "processus infundibuli" (neural lobe) in *Columba* is hollow and that its cavity is continuous with the third ventricle. This statement has later been repeated and verified many times for other birds by investigators, whose main object was an investigation of the adenohypophysis (see literature on pp. 27—29).

Müller's histological notes were later completed by Haller (1898) who was able to observe that the "processus infundibuli" in *Gallus* and *Emberiza* is more folded and complicated than in reptiles and that it consists of ependyma, glia cells, and nerve fibres as in the reptiles.

Gentes (1907 b) surveyed the structure of the pituitary in all vertebrate classes and concluded that the saccus vasculosus, which is a distinct structure in teleosts and selachians, is quite absent in birds. In spite of this Tilney (1912, 1915) states that the saccus vasculosus is present in *Gallus* and *Columba*, but he has been repeatedly contradicted, e. g. by Pokorny (1926). Later (1938) Tilney maintained his idea about the relation between the neurohypophysis and the saccus vasculosus, and this will be discussed in chapter XI.

Herring (1908, 1913) writes that the neural lobe in *Gallus*, *Sturnus* and *Turdus* is made up mainly of ependyma and of a gelatinous granular matter. The origin of this neurohypophyseal colloid has later been discussed extensively, but the material was usually non-avian (compare Collin and Stutinsky, 1949). In 1926 Collin extended his idea concerning the transport of colloid from the pars distalis to the neurohypophysis to apply also to avian material in a paper on *Gallus* and *Anas*. The present knowledge of the structural relationships in birds makes it difficult to maintain this hypothesis (chapter XII). Collin pointed out that the neural lobe is hollow with thin walls in *Gallus* whereas it is compact in *Anas*. He also briefly described the appearance of the glia cells and the perivascular spaces around the capillaries of the neural lobe.

De Beer (1926) noted that the neural lobe is bifid at its posterior end in *Anas*, a statement of considerable interest since such a tendency to paired development can be found in other vertebrates and in avian embryos.

Among the more recent publications I wish to mention particularly the works of Shanklin (1944) and Griffiths (1938). Both authors describe the pituicytes in the neural lobe of *Gallus*. Griffiths describes them as variable in shape, often with very long processes which terminate on connective tissue septa and blood vessels. He also observed the difference between primitive walls and emigrated tissue in the organ. Shanklin's work is discussed on p. 185.

The most complete investigations of the histology of the neurohypophysis of birds seems to be those of Romeis (1940, p. 471). He found a rich system of nerve fibres in the neural lobe of *Columba* and *Gallus*. The fibres form thick layers around the diverticula of the recessus infundibuli, but are less abundant at some distance therefrom. In these peripheral areas there are also islands without nerve fibres around the blood vessels of the same type as in the human hypophysis.

The nerve fibres in the avian pituitary was discussed by Drager (1945) and Green (1951).

A valuable contribution is also the morphological study of Obussier (1948). He found a considerable intraspecific variation in the appearance of the neural lobe in *Anas* and *Gallus*. In general, however, the recessus infundibuli is better preserved and the walls thinner in the latter species.

The general problems concerning the structure and function of the neurohypophysis in mammals have been reviewed e. g. by Collin and Stutinsky (1949), and Heller (1950), and a few remarks concerning the conditions in birds are given by Benoit (1950). Of particular interest is Gersh's (1939) description of parenchymatous glandular cells in the neurohypophysis of *Columba* and *Gallus*. He believes that these cells are responsible for the production of the antidiuretic hormone. The main characteristic of the cells is their content of osmophilic granules, which were observed also in vitro, and they are evidently identical with the cells called pituicytes and "glia" cells resp. in the neural lobe and the eminentia mediana.

General Shape and Subdivisions

The appearance of the neurohypophysis in a number of adult birds is shown in fig. 6, and additional aspects are illustrated in figs. 1—5. It can be seen that the gross anatomy varies considerably from one species to another, and I can confirm Obussier's (1948) statement that also the intraspecific variations are rather great. In general, however, it is possible to distinguish the three subdivisions described in the preceding chapter. The *neural lobe* is always distinct, and as it is broadest near the base it is invariably sharply demarcated from the *infundibular stem*. The latter is never very long in birds, and must be characterized by its morphological features as the tubular projection of the diencephalic wall which carries the neural

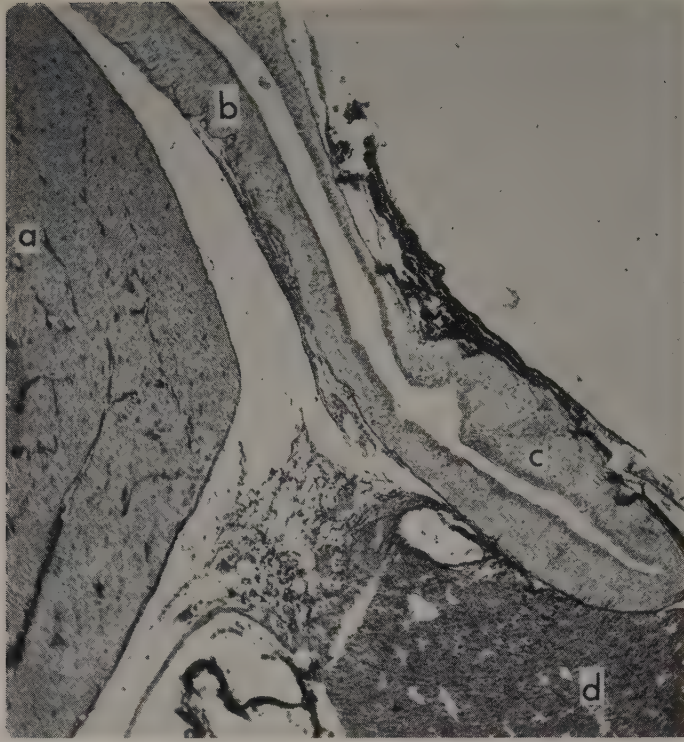


Fig. 108. *Apus apus*. Median section of the neurohypophysis. a = chiasma opticum, b = eminentia mediana, c = lobus nervosus, d = pars distalis. — Bouin, paraffin 6 μ , azan. 85 $\times$.

lobe. In some birds as *Larus*, *Anser*, *Anas*, *Somateria*, and *Gallus* the stem is also morphologically distinct from the eminentia mediana, but in the majority of species the latter limit is rather vague. In median sections of *Apus* it is even impossible to delimit the different portions (fig. 108). The narrow and tubular hypothalamus passes down behind the chiasma and has developed an eminentia on its rostral surface. This narrow tube is directly continuous with the infundibular stem, and as the lateral dilatations of the neural lobe are invisible in a median section also this part appears to be indistinct. The *eminentia mediana*, finally, is the ventral or rostro-ventral wall of the diencephalon from the chiasma to the infundibular stem. Its lateral limits can be stated only if its histological and vascular pattern is considered. The histological appearance of the stem is so closely related to that of the eminentia that it is necessary to describe the two parts together.

The lumen in the neurohypophysis, the so-called recessus infundibuli, extends throughout the infundibular stem in all birds. Its development in the neural lobe varies within wide limits. If the gland has been formed by diffuse emigration of cells as in *Riparia* (fig. 102) the lumen is restricted to the very base of the nearly compact lobe. If, on the other hand, hollow buds have played a considerable rôle

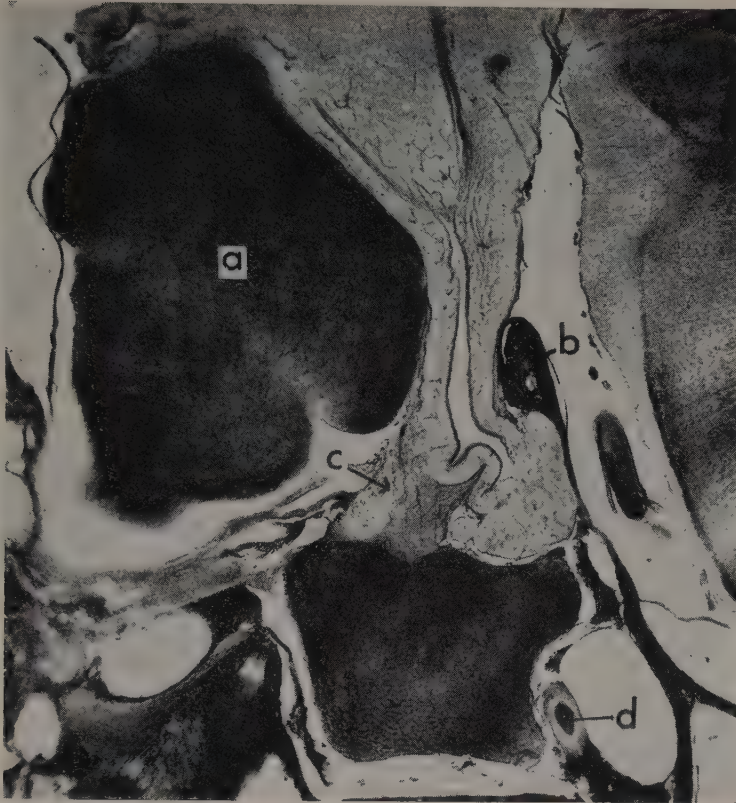


Fig. 109. *Pelecanoides magellani*. Median section of the pituitary region. a = chiasma opticum, b = dorsum sellae, c = pars tuberalis, d = intercarotic anastomosis. Note the compact neural lobe and the vertical eminentia mediana. — Bouin, celloidin 60 μ , Mallory's phosphotungstic acid haematoxylin. 23 $\times$.

for the formation of the organ as in *Gallus*, the recessus is wide and the entire organ may have the shape of a thin-walled, wrinkled sac. The different types of neural lobes will be mentioned under the description of the histological structure.

The Eminentia mediana and the Infundibular Stem

General Appearance

The eminentia mediana is characterized by its intimate relation to the primary capillary plexus of the hypophysioportal system and by its histological features. In most birds it is developed as a more or less horizontal floor of the third ventricle. It then extends from the optic chiasma rostrally to the infundibular stem caudally, and it never reaches far upwards the sides of the hypothalamus but is restricted to the most ventral parts of the wall. In *Anser* (fig. 1) and *Anas*, how-

ever, it does not reach the posterior limit of the chiasma but stops at some distance from it. In some birds with an exceptional appearance of the hypothalamus the eminentia may occupy another position. Thus, in *Apus*, *Pelecanoides*, *Sephanoides* and some passerine birds the transversal cleft between the chiasma and the eminentia is very deep, and the eminentia is therefore more vertical, forming the posterior wall of this cleft (fig. 6, 108 and 109).

The external surface of the eminentia is smooth in most birds or there may be some shallow furrows which lodge the capillaries on the surface. In some large species of birds, especially *Struthio* and *Anser*, these furrows are very deep and separate lobe-shaped ridges which run in a predominantly longitudinal direction. In *Spheniscus* the eminentia is still more complicated and the entire wall is folded in both my specimens (fig. 6: B).

In all birds it is possible to distinguish three layers in a section through the median eminence (fig. 110), and as these layers will be discussed extensively in the following it is necessary to introduce a terminology for them. It should be mentioned already here, however, that certain modifications of this general pattern exist in the peripheral parts of the eminentia, where transitional zones to other hypothalamic regions are present.

1. The *stratum ependymale* or *ependymal layer*, which consists of the cell bodies of the ependyma, contains very few and scattered nerve fibres and sometimes also a few scattered nerve cells. The processes of the ependymal cells run through the other two layers to the external surface.

2. The *stratum fibrosum* or *fibre layer*, is dominated by the mighty bundles of coarse nerve fibres which run from rostral or rostro-lateral directions towards the infundibular stem. The bundles form a distinct tract which will be called the *tractus hypophyseus*. The bundles are separated from each other by the processes of the ependymal cells. This layer often contains also a number of glia cells, most of which send their processes towards the external surface like the ependymal cells.

3. The *stratum glandulare* or *glandular layer*, which occupies the superficial part of the wall. The glandular layer is characterized by the partial or complete absence of coarse nerve fibres and cell nuclei and is traversed by numerous fine processes of the ependymal and glia cells. All these filaments run parallelly towards the external surface and attach to the intima piaie by broad, dilated ends ("vascular feet").

Ependymal Cells

The ependymal cells can often be studied in preparations stained in haematoxylin, acid fuchsin, and azan, but their processes can be clearly distinguished from nerve fibres only if the azan staining is preceded by Bodian impregnation of nerve fibres. The clearest pictures were obtained after fixation in alcohol-formalin-acetic acid, injected from the carotis. Also Golgi preparations of *Columba*, *Pica*, and *Gallus* proved valuable for studies of ependymal cells including their processes.

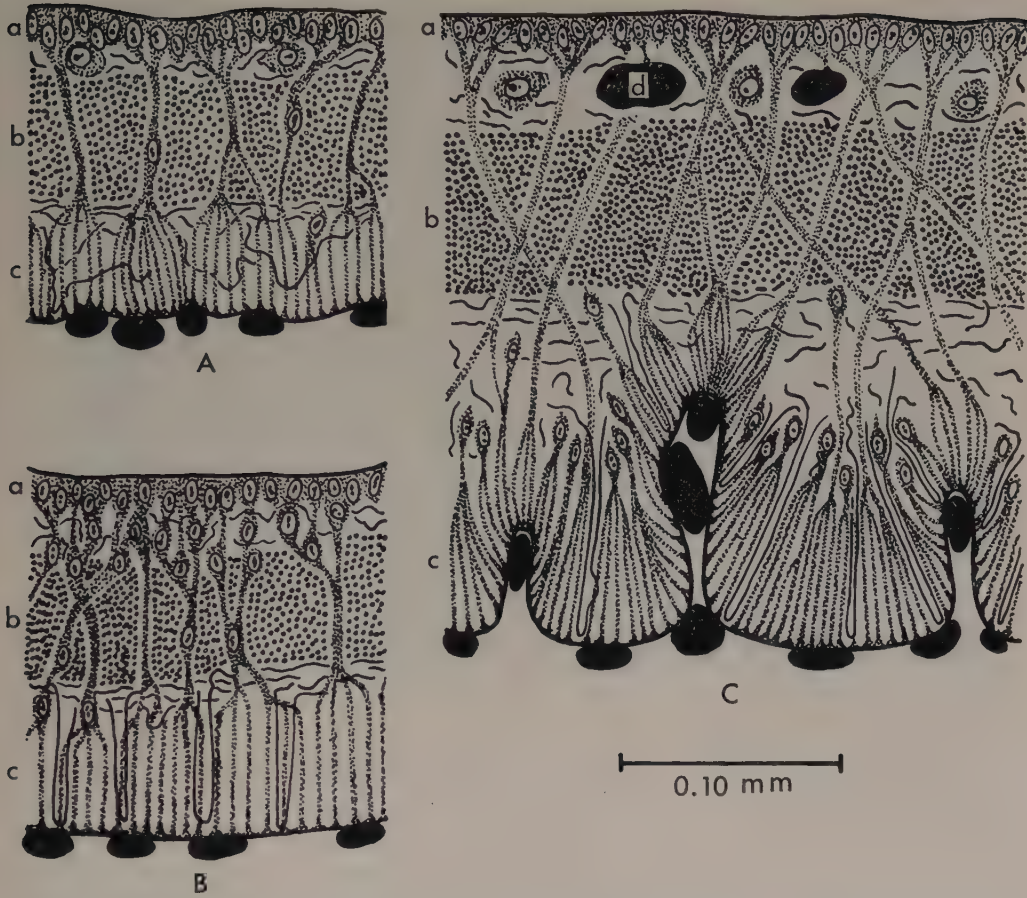


Fig. 110. Diagrams showing a cross section of the eminentia mediana in *Coragyps atratus* (A), *Columba livia* (B), and *Anser anser* (C). Ependymal and glia cells are stippled, blood vessels are black, and nerve fibres are indicated by black lines, or, in cross section, by large dots. a = ependymal layer, b = fibre layer, c = glandular layer, d = non-portal, hypothalamic capillaries. — A and C from 8μ sections, fixed in Bouin and stained in Bodian — azan. B is a synthesis of several slides. The magnification is the same for all figures (note scale to the right).

The cell bodies of the ependymal cells form a continuous layer along the surface of the ventricle. In *Anser* and *Branta* it could be stated that each cell carries a flagella from its inner surface, but such flagellae could not be found in other birds. In the said geese the basal granules of the flagellae are particularly distinct after Bodian impregnation, but the filaments are short and not always clearly visible owing to the presence of precipitated matter from the ventricular liquid. Also single multiciliated cells were found among the uni-flagellated ones in *Anser*. Other birds seem to lack flagellae in the eminentia region, but the microscopical picture is obscured by precipitates from the ventricular liquid and by a

row of vacuoles along the ependymal surface, so the statement is not definite in all cases. The said vacuoles, usually one for each ependymal cell, appear quite clear in the fixed and stained preparations whereas the surrounding liquid contains stainable precipitates.

The ependymal cells emit one or a few coarse processes, which are directed towards the external surface. Though it is difficult to ascertain whether all ependymal cells behave in this way I have found nothing to disprove it. The coarse ependymal processes never branch within the fibre layer, but are packed together into vertical membranes which separate the bundles of nerve fibres (fig. 110). When the processes reach the glandular layer they suddenly branch into numerous fine filaments, which run straight through this layer to the external surface where they end with conical vascular feet. Thin sections indicate that these dilated ends of the fibres fuse and form a continuous layer of plasm under the intima piaie. The shape of the ependymal cells varies from one species to another within the limits given by the above description, but no fundamental differences were found.

The Fibre Layer and the Glia Cells

The course of the nerve fibres of the eminentia will be described in detail in chapter X, and only their relation to the glia cells of the wall will therefore be considered here.

In the simplest case, e.g. in small passerine birds, *Apus*, *Coragyps*, some specimens of *Anas*, *Larus ridibundus* and *argentatus*, very few cells have left the ependymal layer and invaded the fibre zone (fig. 110 A, 111). They are irregularly scattered throughout the fibre layer. Most of them emit one or a few coarse processes which branch in the glandular layer and attach to the external surface as described for the ependymal cells. Some of them also send a single process towards the inner surface where it terminates among the cell bodies of the ependymal cells. This only means that the cells are connected with their point of origin in the ependyma. A small number of glia cells are bipolar and send their processes along the fibres of the tractus.

In most birds the number of invaded glia cells is considerable, and they are then more or less concentrated at a certain level in the wall. Thus, in *Diomedea* and *Columba* (fig. 110 B, 112) there is a distinct and mighty layer of glia cells on the inner (ventricle-facing) side of the fibre layer, between it and the ependyma. In the majority of birds the glia cells have passed the tractus and become concentrated along its outer surface. In the most complicated eminentiae, e.g. in *Anser* and *Branta*, where the surface of the eminentia is folded, the glia cells are concentrated at the base of the ridges between the furrows, so the layer is no longer continuous (fig. 110 C).

I have used the term glia cells in the above description for the free cells in the eminentia. Apart from their localization, however, they are very little different from the ependymal cells which line the ventricle. The processes of both these

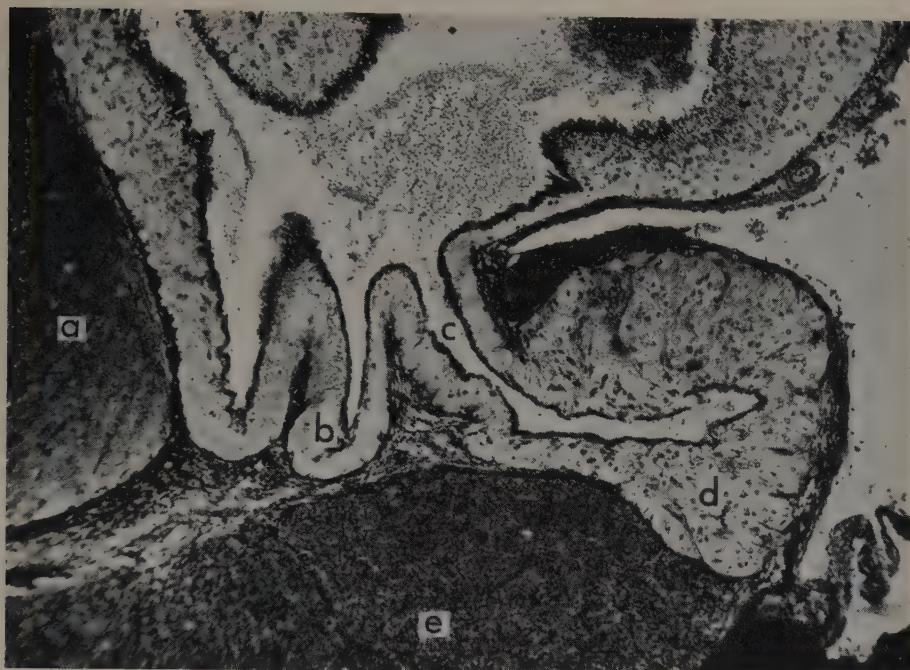


Fig. 111. *Turdus pilaris*. Median section of the neurohypophysis. a = chiasma opticum, b = eminentia mediana, c = infundibular stem, d = lobus nervosus, e = pars distalis. Note the absence of internal cells from the wall of the eminentia. — Bouin, paraffin 6 μ , azan. 95 $\times$.

cell types behave in the same way and show the same staining reactions. In some smaller bird species, e. g. in *Turdus pilaris* (fig. 111), the glia cells are very rare, and it should be mentioned that they are completely absent in the eminentia of some lizards in my collection. In such species which practically or completely lack free glia cells the function of these cells must be exerted by the morphologically similar ependymal cells which are the only additional cell type in the wall. Further it will be shown below that the free glia cells of the eminentia are structurally and probably also functionally related to the free cells of the neural lobe, which are usually called pituicytes.

Also the pituicytes may be completely replaced by ependymal cells in some bird species. It is thus far from clear whether the free cells of the eminentia are essentially different from pituicytes or not, but I have used the term glia cells here in order to avoid confusion.

The Glandular Layer and the Structure of the Surface

The very characteristic appearance of the glandular layer mainly depends on the dense system of fine ependymal fibres which run to the surface, on the absence of coarse nerve fibres and cell nuclei. In a great number of preparations the fine



Fig. 112. *Columba livia*. Median section through the eminentia mediana, showing the localization of the glia cells near the ependyma, the tractus hypophyseus and the superficial decussation. — Alcohol-formalin-acetic acid, paraffin 10 μ , Bodian — azan. 500 $\times$.

ependymal fibres are difficult to observe and the glandular zone appears to be filled with a floccular, stainable colloid. However, when the specimens have been fixed by injection from the carotis or when other precautions grant a rapid penetration of the fixative, the azan staining always reveals them. They are especially distinct if the azan staining is preceded by a Bodian impregnation, because the interstitial colloid of the glandular zone is partly removed during this process (fig. 112).

Under low magnification this layer often appears as a dark red or violet margin in the azan preparations, partly because of the high stainability of the ependymal processes, partly because of the presence of a stainable colloid in the interstitia. This interstitial colloid, which is present also in perfectly fixed preparations, is not identical with the matter which is secreted by the neurons of the supraoptico-hypophyseal tract and which can be stained with chromic haematoxylin of Gomori. The "Gomori-positive" substance is abundant in the fibre bundles of the tractus and to a very restricted extent also in the rostral part of the glandular layer of birds, whereas it is absent from its caudal part (cf. p. 227—228).

The coarse fibres of the tractus hypophyseus do not enter the glandular layer of the eminentia, with a few exceptions in the rostral part of the organ. However, when successful impregnations have been made (either according to Bodian 1936 or to Palmgren 1948) the glandular layer can be shown to contain a specific innervation

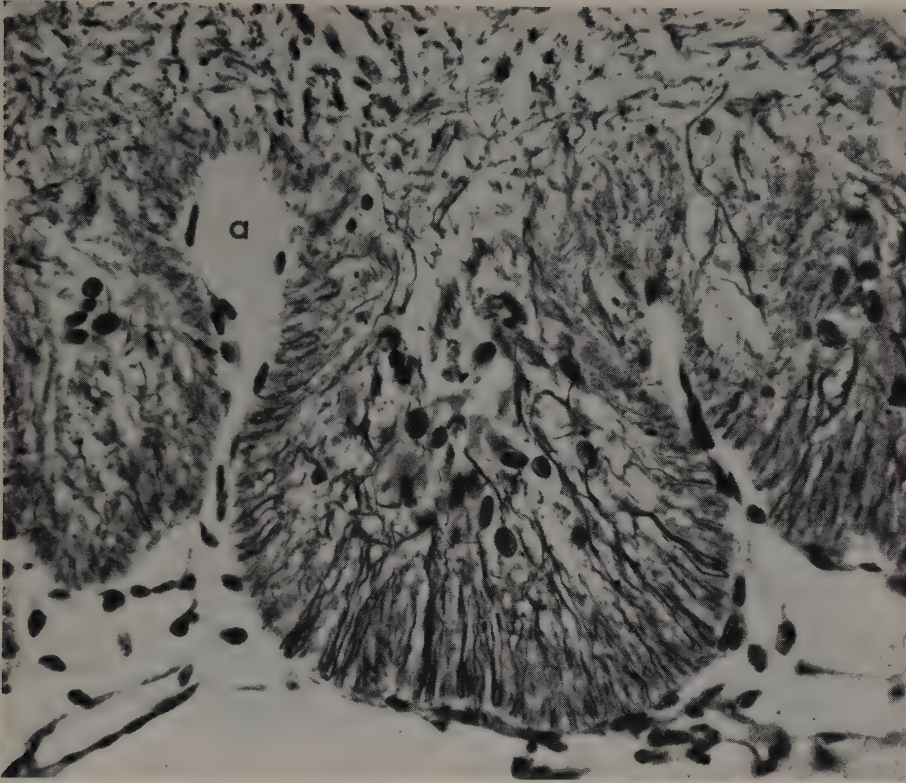


Fig. 113. *Anser anser*, transversal section of the superficial part of the eminentia. The nerve loops in the glandular layer are distinct, and also the deep furrows on the surface, which contain the portal capillaries (a). — Alcohol-formalin-acetic acid, Bodian impregnation. 500 X.

which has its origin in a plexus of fine fibres at the base of the layer immediately outside the fibre layer. In the simplest case (some specimens of *Columba livia*) the relatively narrow glandular layer contains a system of fine irregular threads which are more or less parallel to the external surface. Some of these fibres tend to form loops with the bending-point near the intima piae. In the specimens of *Anas* and *Branta* and in some pigeons these loops are more numerous and regular, and in some parts of the eminentia of *Anser*, *Coragyps* and *Larus* there is a real wood of long and slender loops which run towards the external surface parallel to the ependymal fibres (fig. 113). The fibres start from the fibre plexus at the base of the glandular layer and run along the ependymal fibres to the external surface, but just near the intima piae they suddenly turn and pass back again the same way. Though these fibres sometimes form varicose swellings it could never be ascertained that they terminate in the glandular layer, nor do they penetrate the intima and leave the organ.

As already mentioned the eminentia is covered by the extremely dense capillary net of the hypophysio-portal system. If the tuberalis is well developed the

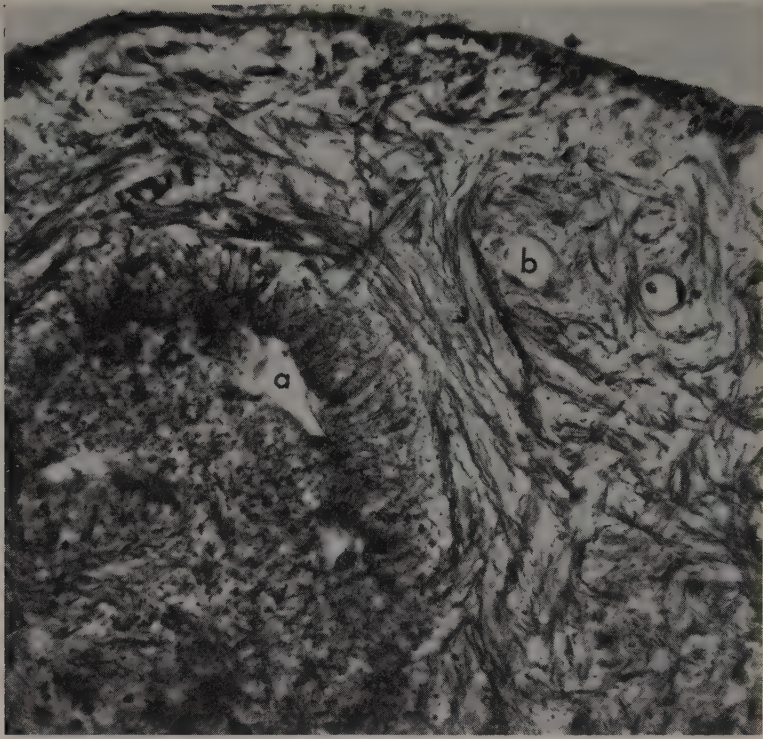


Fig. 114. *Branta leucopsis*. Sagittal section of the eminentia mediana. Some portal vessels (e. g. "a") to the left have an exceptional situation within the wall. They are surrounded by stainable glandular zones in contrast to the common hypothalamic capillaries (b). — Bouin, paraffin 8 μ , Bodian — azan. 400 $\times$.

capillaries are situated between the epithelium and the intima piae. When the surface of the eminentia is smooth these capillaries are flattened and attach with one side to the intima piae. It is not possible to observe more than one reticular membrane between the vessel and the nervous tissue: the perivascular "adventitia" and the intima piae have fused completely. The blood of the capillaries is thus separated from the glandular layer only by the endothelium of the vessel, by one thin reticular membrane and perhaps by a continuous glia sheath formed by the vascular feet inside the intima piae. There are thus favourable conditions for exchange of substances between the blood and the glandular layer. Between and outside the capillaries there are also collagenous connective tissue fibres (pia mater), which become abundant towards the periphery of the eminentia.

In most birds the superficial capillaries have sunk down into shallow furrows on the surface of the eminentia, and in *Struthio*, *Branta*, and *Anser* these furrows are so deep that some capillaries seem to run through the tissue of the eminentia. Even if some of the superficial capillaries may have sunk deep into the eminentia they are always surrounded by a typical glandular zone. In this respect they differ

sharply from the non-portal capillaries near the ependyma, which belong to the common hypothalamic plexus (fig. 114).

With some exceptions in a few large birds such as *Anser*, *Struthio*, *Diomedea* and *Branta* the median caudal parts of the eminentia are supplied only by the superficial portal capillaries. The lateral and rostral parts, and in the said birds the entire eminentia, contains another system of capillaries which is almost completely independent of the portal system and is connected with the hypothalamic net (chapter XIII). These vessels are situated between the ependyma and the fibre layer in the inner parts of the wall. They have the common appearance of brain capillaries such as they were described in man by Held (1909). There are two more or less distinct membranes around the vessel: the perivascular adventitia and the intima piae, and between these membranes there are sometimes large spaces ("Virchow-Robinscher Lymphraum"). No glandular zones are present around these vessels, but the glia processes which attach to the intima piae are comparatively few and irregular. It seems indisputable that the great difference in the structures around the portal and non-portal capillaries indicates a different function.

The exceptional structure of the glandular layer, especially the dense system of parallel ependymal fibres and the peculiar innervation indicates that the layer has a function different from that of superficial glia zones elsewhere. The accumulation of stainable colloid and the extremely rich vascularization support the opinion that this function is glandular. The secretions must certainly be washed away by the portal capillaries, for other capillaries are lacking in parts of the eminentia of most birds. It is of course possible that some secretory products arrive into the ventricle, but the intimate contact between the glandular layer and the portal capillaries on the one hand and the distance to the ventricle on the other hand makes it probable that this proportion is small. Note, however, the vacuoles in the ventricular liquid described on p. 192.

The Delimitation of the Eminentia and the Structure of its Marginal Zones

It was said above that the eminentia mediana is defined as the region covered by the primary capillary plexus of the hypophysioportal system. A great number of preparations show that this area is coextensive with the very typical glandular layer. The pars tuberalis, on the contrary, regularly extends far beyond the eminentia, especially upwards of the sides of the hypothalamus behind the chiasma.

The histological limit of the eminentia is rather sharp rostrally, where it extends to or, in geese and ducks, to near the chiasma. These most rostral parts are, however, less typical since they contain a diffuse layer of large ganglion cells in the zone between the tractus fibres and the ependyma. The nerve cells undoubtedly correspond to the caudal end of the "nucleus a" of Rendahl (1923), a nucleus which is very distinct in embryos (fig. 94). Caudally the delimitation of the eminentia is less distinct for its structure gradually changes into that of the infundibular stem, and the two parts are not very distinct in most birds.

Laterally, where the eminentia structure meets the general hypothalamic pattern, we find another transitional zone. Here the glandular layer is distinct under the intima piaie and also a fibre layer is present. The wall is, however, complicated by the nuclei tuberis (Kuhlenbeck 1937) which extend into the eminentia region between the ependyma and the fibre layer. In a few birds the nerve cells even extend as a diffuse layer near the ependyma to the median line of the eminentia, so that the nuclei tuberis of both sides are completely continuous. This was observed in *Anser*, *Anas*, and *Phalacrocorax*, but in other birds the central parts of the eminentia completely lack nerve cells. It can be observed that the extension of the nerve cells within the eminentia coincides with the extension of the hypothalamic capillaries. It is, of course, natural that the capillary bed of the nuclei follows the latter when they extend ventrally, but the observation is noteworthy, for it emphasizes the independence of the portal and hypothalamic vascular systems.

Exceptional Structures in the Eminentia

It was mentioned in chapter III that the epithelial cells of the pars tuberalis often, perhaps regularly, invade the eminentia in the Procellariiformes. Diffuse emigration of nervous matter leading to the formation of tongue-like secondary lobes from one or more openings in the surface of the eminentia has been observed in a few specimens (of *Pica*, *Corvus*, *Diomedea melanophrys*, *Spheniscus*). Perhaps some of these secondary lobes have been formed from diverticula similar to those described in embryonic chicks (p. 170). They show, anyhow, that the potencies for diffuse emigration are not entirely restricted to the neural lobe.

Probably some kind of exceptional proliferation plays a rôle also in the development of the complicated eminentia in the geese. This is indicated by the presence of scattered fibres of connective tissue within the glandular layer and sometimes also in the fibre layer of the organ. Gömöry and azan preparations clearly show that these irregular fibres are of similar kind as those which are developed from included mesenchymatic elements in the secondary tissue of the neural lobe. Yet the mixing of mesenchymatic and nervous elements can hardly have been so radical as during the development of the latter organ, for there is a sharp limit between the nervous elements and the connective tissue at the intima piaie.

The bulb-shaped nerve endings which have been found repeatedly in the tractus layer and near the ependyma in the eminentia of *Anser* and *Branta* might also be regarded as exceptional structures, because I have searched for them in vain in Bodian preparations of other species. They are of the same types as the nerve endings in the neural lobe and are described together with them (p. 228 ff.).

The Infundibular Stem

The structure of the infundibular stem is essentially the same as that of the eminentia. The ependymal layer along the ventricle is always present. The fibre layer is thicker in the stem, for here the tractus fibres are more concentrated.

This applies to the ventral and lateral walls of the stem, for the fibres do not shift over to the dorsal (caudal) side of the stem immediately at its base, and the fibre layer is therefore thinner or almost absent there. The glandular layer, in typical cases at least, is little developed although it can be distinctly recognized. Nerve cells have never been found in the stem, nor are there any internal capillaries in the wall. The capillary net on the surface is not so dense as that of the eminentia, but the two systems are completely continuous. The said differences between the stem and the eminentia exist mainly in birds such as the Anseriformes, which have a long and distinct stem, but in many other birds the differences are hardly recognizable. The dorsal (caudal) wall of the stem is, however, always thinner with a poorly developed glandular zone and a thin tractus layer in the basal part.

Histological characteristics thus do not justify a distinction between the median eminence and the stem, so the maintenance of the latter term must be supported by embryological and morphological facts only. In his monograph on the innervation and vascularization of the hypophysis Green (1951) uses the term infundibular stem or, together with the tuberalis, "hypophysial stalk" for purely descriptive purposes and does not regard it as a comparative anatomical unit. The fact that it is formed by the base of the saccus infundibuli in the embryos and therefore is closely related to the neural lobe makes me hesitate, however. It would be unnatural to include the stem in the term neural lobe because of its histological affinities to the eminentia.

The Lobus nervosus

General Structure

The neural lobe is the most variable part of the avian pituitary, both as regards gross structure and histological details. It is flattened dorso-ventrally and its breadth usually exceeds its length and height. When viewed from above it often looks heart-shaped or kidney-shaped, but in some cases it is so broad that even these comparisons are inadequate (fig. 102). The surface is irregularly lobular, and in some specimens (of *Struthio*, *Anas*, *Caprimulgus*) there is a bifid posterior extremity indicating the paired tendency of the organ in embryos (cf. p. 180).

The different modes of embryonic development described in chapter VIII can be distinctly traced also in adult birds, if the histological details are studied.

The best preparations for a general investigation of the neural lobe proved to be 8–10 μ paraffin sections, first impregnated according to Bodian and then stained in azan. The best fixative for this treatment was alcohol-formalin-acetic acid, but also Bouin and Susa gave good results. In such preparations the nerve fibres are black, the ependymal cells and glia cells (pituicytes) with their processes red, and the connective tissue elements blue. The silver carbonate methods of Rio-Hortega proved less useful, for the structure of the organ with its delicate and complicated walls etc. is usually destroyed if frozen sections are prepared, and when applying this method for paraffin sections according to Shanklin (1943) I never got perfectly clear pictures although they showed the essential features of the pituicytes. The argyrophilic reticular fibres were perfectly demonstrated in preparations impregnated with

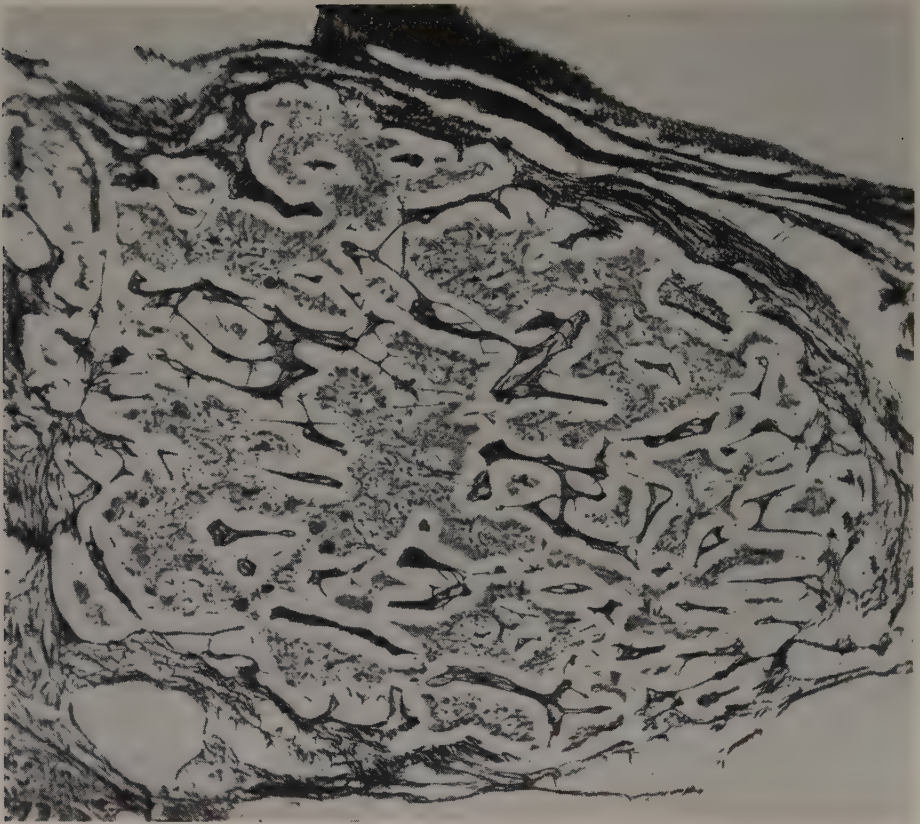


Fig. 115. *Anser anser*. Sagittal section through the lateral parts of the neural lobe, impregnated with silver to show the connective tissue elements. The argyrophilic components stand out as distinct black fibres, surrounded by white fields. At such places where no argyrophilic structures are present the silver is precipitated as a more greyish, almost homogeneous cloud. — Alcohol-formalin-acetic acid, paraffin 8μ , impregnation according to Gömöry. $95\times$.

silver according to Gömöry, but could be seen as blue structures also in azan preparations. The nerve fibres are very clear after Bodian impregnation, but also the silver impregnation method of Palmgren (1948) proved very useful. A great number of other nerve impregnation methods were tried but did not prove superior to these two (cf. p. 209). With the exception of delicate pituicyte and ependymal processes most structures in the neural lobe could be studied also in azan preparations.

The features distinguishing the primitive, non-emigrated parts of the neural lobe from the secondary tissue are, as will be clear from the embryological development:

The primitive walls consist of an ependymal layer near the lumen, a fibre layer, and a relatively narrow glandular zone near the surface. The connective tissue and the vessels are restricted to the external surface (fig. 116, 119 A).

The secondary tissue, on the contrary, is compact and contains both internal vessels and fibres or irregular bodies showing connective tissue reactions (fig. 115).

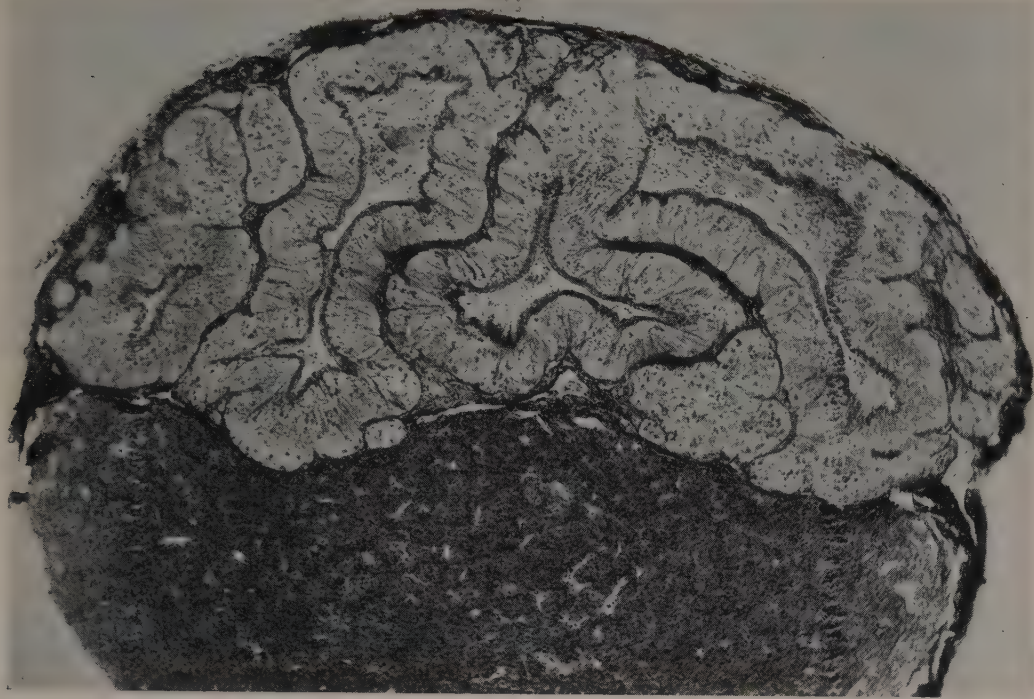


Fig. 116. *Phasianus colchicus*, ♂. Cross section through the posterior end of the pituitary, showing the primitive appearance of the lobus nervosus. Very little secondary tissue is present and the gland is a wrinkled sac with thin, primitive walls. — Bouin, paraffin 8μ , azan. $90\times$.

The nerve fibres and the cells (pituicytes) are more irregularly distributed, but usually rearranged so that a perivascular space, free from coarse nerve fibres and cell bodies and traversed by pituicyte processes is formed around the capillaries (fig. 119). The secondary tissue is often indistinctly delimited from the capsule of coarse connective tissue which surrounds the lobe and there is usually a zone with an increasing amount of collagenous fibres near the surface. In the primitive walls the superficial membrane is very sharp.

In a few cases, especially in the passerine birds and the birds of prey, some primitive walls have increased in thickness and are intermingled with mesenchymatic derivatives in the peripheral zones, so the result looks somewhat intermediate between primitive wall and secondary tissue.

The Different Types of Neural Lobes

Though the individual variations in the neural lobe are large there are unquestionably also certain features of taxonomic significance. The differences mainly concern the degree of complication of the lumina and the relative amount

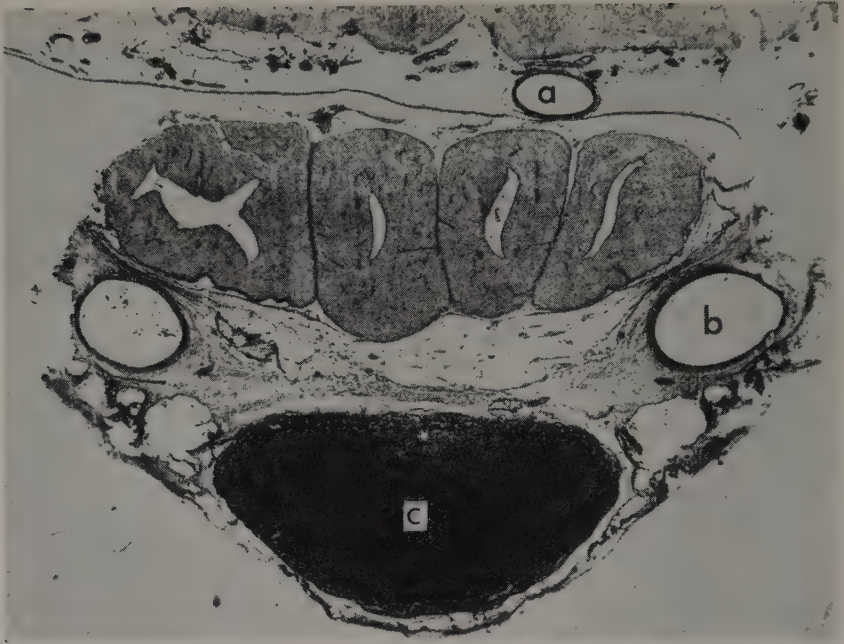


Fig. 117. *Struthio camelus*, young. Cross section through the posterior end of the pituitary, showing its paired development. There are four distinct diverticula of the lumen with secondarily thickened walls. a = ramus posterior of the carotid, b = carotis cerebralis, c = pars distalis. Bouin, paraffin 8 μ , azan. 20 $\times$.

of secondary tissue. I have tried to group the examined birds into four categories according to the appearance of the neural lobe.

Type 1. Diomedea, Procellaria, Gallus, Lagopus, Phasianus, Lyrurus, Strix, Asio. The neural lobe in these species is very simple from a morphological point of view. It is formed almost exclusively by hollow buds and has very thin primitive walls and a complicated and large lumen (fig. 116). The amount of emigrated secondary tissue is insignificant in most specimens of these species and occurs only in a few restricted areas. Practically the whole lobe is made up of primitive walls with the ependyma as the only kind of cells. Pituicytes are rare and occur only in the small portions of secondary tissue. The paired condition established in young embryos by the development of primary branches is hardly visible in the adult because it is hidden by the numerous hollow buds (fig. 116).

Though this simple structure of the neural lobe seems to be restricted to the three orders Strigiformes, Procellariiformes, and Galliformes, it has no high taxonomic significance. In the last two orders we also find highly complicated neural lobes (*Pelecanoides, Priocella, Perdix*, fig. 118, some fowls).

Type 2. The majority of birds, including *Struthio, Oceanites, Nycticorax, Charadriiformes, Columbiformes, Falconiformes, Psittaciformes, Piciformes, Apodiformes, Passeriformes*.

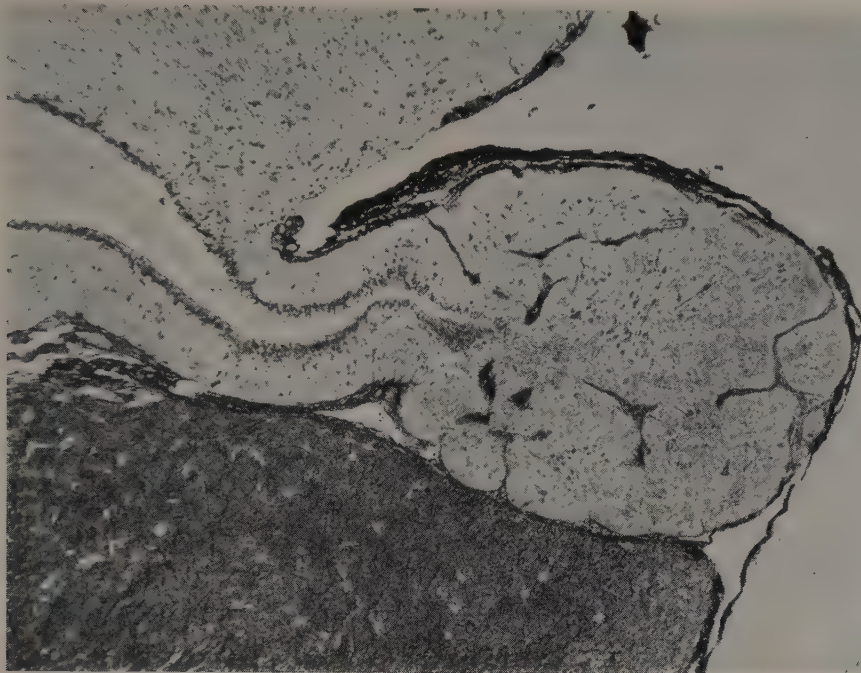


Fig. 118. *Perdix perdix*. Median section of the posterior part of the pituitary, showing the compact type of neural lobe. The lumen stops at the base of the lobe. The infundibular stem is distinct in this specimen. Bouin, paraffin 6 μ , azan. 85 $\times$.

In these birds there is always a distinct central lumen near the base of the lobe, which extends laterally as a pair of blunt diverticula. The lumen may be more or less extensive; the lateral branches are sometimes divided into two or three diverticula each (distinct in *Struthio*, fig. 117, *Coragyps*, *Nycticorax*), but the walls are thick and do not show the appearance of primitive walls. In small passerine birds as *Emberiza* and *Parus* the thickening is slight and only the superficial zones have been intermixed with mesenchymatic elements. In most species, however, the thickening is considerable and secondary tissue makes up most of the gland.

The paired condition with primary branches is often distinct in the lateral development of the lumen, but the branches are fairly variable. In some birds as *Struthio*, *Turdus pilaris*, *Phylloscopus* and *Parus* there are few additional diverticula, but they are more common in *Columba*, *Coragyps*, *Pica*, etc.

Type 3. Pelecanoides, Priocella, Phalacrocorax, Anseriformes, Larus, Perdix, Cuculus, Caprimulgus.

The neural lobe is not very different from that described as type 2, but the amount of secondary tissue is greater, giving the organ a compact appearance. The lumen is restricted to the very base of the gland and a pair of lateral diverticula (primary branches) run along the rostro-lateral surface. One or a few

median diverticula may also be present. Primitive, non-proliferated walls are present only between the lateral lumina and the surface, and scattered fragments of unaltered walls can also be found around other lumina, if any, within the lobe. The pituicytes are very numerous and form the prevailing cellular element of the lobe, except in *Perdix* in which these cells, too, are few (fig. 118). The ependymal cells have, of course, a restricted distribution.

Type 4. Spheniscus.

In *Spheniscus* there is a neural lobe which appears to be compact. There are no distinct lobuli separated by septa, but only scattered membranes and connective tissue fibres within the gland. However, the lumen continues into all parts of the gland as very narrow channels lined by ependyma. In fact, there are almost as many ependymal cells in the neural lobe as there are pituicytes. Evidently the number of buds from the saccus has been very large, but the buds have fused and their superficial membranes have partly disappeared. A similar process can be seen in a less advanced stage in the *Diomedea* specimens and *Procellaria*, in which some of the numerous buds have fused and only the lumina remain separate. One of the *Procellaria* specimens is definitely of this type.

The Pituicytes and the Ependymal Cells

The free cells in the neural lobe (pituicytes) and the ependymal cells do not show any differences in staining reactions and are morphologically very much alike. The ependymal cells look like those in the eminentia, but their processes are not as excessively branched as there. Their processes attach with conical "vascular feet" to the superficial membrane of the primitive walls, or to the membrane around vessels in the secondary tissue if the gland is of a more compact type (fig. 119 A).

The pituicytes are irregularly star-shaped, in most species with 2—4 processes, which attach in the same way to connective tissue fragments and to the intima piae around capillaries in the secondary tissue. There are considerable variations in detail in the morphological appearance of the ependymal cells and the pituicytes, but the above description is valid for all species examined (cf. fig. 119 B). In *Struthio* they are usually bipolar with two processes.

The zone under the intima piae of the primitive walls just as the zone around the capillaries is developed in a way reminding of the glandular zone of the eminentia. There are no coarse nerve fibres nor pituicyte nuclei, and the space is traversed by the processes of the ependymal cells and pituicytes which attach to the membrane with dilated ends. The plasmatic processes are, however, fairly few, if compared with the dense wood of such processes in the eminentia. The primitive walls always have a glandular zone, but the corresponding perivascular spaces in the secondary tissue are more variable. In *Anser*, *Anas*, *Branta*, *Coragyps*, *Phalacrocorax* and *Larus* they are distinct and regular, but they are not equally distinct in other birds. In *Nycticorax* and some passerine birds they are even



Fig. 119. Diagrams showing the structure of the neural lobe of *Diomedea melanophris* (A) and *Anser anser* (B). Ependymal cells (in A) and pituicytes (in B) are dark stippled, blood vessels are black, nerve fibres are drawn as dark, irregular lines. In B the perivascular spaces are light stippled (containing violet colloid in the preparation). Connective tissue elements not considered. — Both figures from 8μ sections, stained in Bodian — azan. $350\times$.

difficult to find at all. This depends mainly on a more irregular distribution of nerve fibres and pituicyte nuclei, whereas the processes of the latter attach to the capillaries in a quite typical way.

Nerve Fibres and Vessels

The tractus hypophyseus forms a thick fibre layer around the lumen at the entrance into the neural lobe. If the lumen is large and complicated this fibre layer is continuous outside the ependyma in the entire gland or as far as the lumen reaches, forming the fibre layer of the primitive walls. In species with a more restricted lumen the tractus breaks up into bundles which pass into the secondary tissue at places apparently corresponding to the emigration points in the embryo (fig. 120). In the secondary tissue these bundles form a tangle of fibres, which occupy the interspaces between the capillaries together with the cell bodies of the pituicytes. No endings could be found in this tangle apart from large bulb-shaped ones described in chapter X.

I have searched intensively for nerve fibres in the perivascular spaces of the neural lobe. In some successful preparations of *Anser*, *Anas*, *Corvix* and *Phalacrocorax* I found irregular, very fine and little stainable fibres there, and a few of these fibres tend to form loops similar to those in the eminentia. The fibres are not very numerous, however, and were searched for in vain in many preparations.

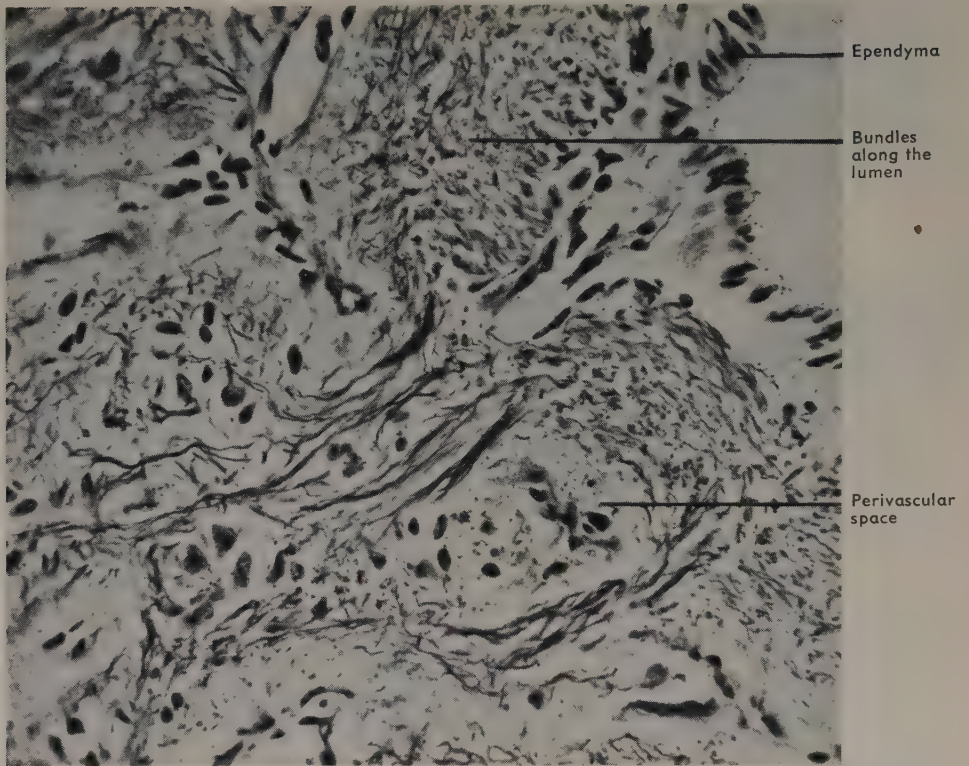


Fig. 120. *Anser anser*. Part of a cross section through the lobus nervosus, impregnated according to Bodian. The coarse nerve bundles around the recessus infundibuli (upper right corner) are distinct, and also fibres radiating from this layer into the secondary tissue. — Bouin, paraffin 8 μ , Bodian, 500 $\times$.

The capillaries have the same structure as in the eminentia with a wall consisting of the endothelium and a reticular membrane which is rarely double in the secondary tissue — it is probably made up only of the perivascular elements since the intima piae has disintegrated. Arteries with a distinct media are regularly present near the posterior end. The vessels in the secondary tissue are often accompanied by strings of collagenous connective tissue.

Interstitial Colloid

The *interstitial colloid* is distinctly stained by the chromic haematoxylin of Gomori in the same specific way as the colloid throughout the route of the tractus supraoptico-hypophyseus (cf. p. 228). It could not be decided whether colloid negative to this stain is present or not. The Gomori-positive colloid, which is visible also in azan preparations, is often distinctly concentrated in the perivascular spaces of the secondary tissue and in the glandular zone of the primitive walls.

Aberrant or Rare Structures

Exceptional aggregates of pituicytes with short processes and rich plasm may form islands of a more compact appearance in any part of the neural lobe in most species. Nerve cells are absent from all neural lobes in my collection with a few exceptions in some preparations of goose. In one of these geese (*Anser anser*, domestic) some sympathetic ganglion cells of the carotic plexus had apparently been included when the diffuse emigration took place, for they are few and situated near the posterior end of the organ. In another specimen there are ganglion-like cells scattered throughout the neural lobe. This latter specimen must, however, be regarded as very exceptional. In two genera (*Spheniscus*, *Phalacrocorax*) there are aggregates of bladder-like cells of the same appearance as those described as "Neuroepithelzellen" from human material by Romeis (1940, p. 426). Shanklin's (1947) description of the "tumorette" cells in the human neurohypophysis also applies perfectly to these cells, especially his fig. 6 is strikingly like my preparations. The content of the large cell bodies is granular and little stainable, but the cells are well delimited and have a distinct nucleus near the periphery.

CHAPTER X

The Innervation of the Hypophysis

Literature

The innervation of the avian hypophysis was practically unknown until the last few years. In Boon's treatise (1938, p. 23) it is mentioned that fibres from the nucleus supraopticus and also from small "tuber cells" may be followed to the neurohypophysis in the ostrich (own observation?). The papers dealing with the structure of the avian diencephalon (Rendahl 1924, Huber and Crosby 1929, Kuhlenbeck 1937) are valuable for identification of nuclear masses but pay little attention to the hypophyseal fibres, neither does Kurotsu (1935) describe them in his detailed account of the nucleus magnocellularis periventricularis.

The first to try a survey of the hypophyseal fibres in birds was Drager (1945), who worked with the chick. He found a prominent hypothalamico-hypophyseal fasciculus, which mainly terminates in the neural lobe. A few fibres are said to pass into the pars tuberalis, which also receives fibres of autonomic origin from the carotid plexus. The hypothalamico-hypophyseal fasciculus was traced back to the region dorsally of the chiasma. Drager found no nerve fibres in the pars distalis (cf. also Drager 1944).

Green (1951) in his valuable monograph on the vessels and nerves of the pituitary carries our knowledge one step further. He traced the "tractus hypophysius" in the same direction as Drager and found that the majority of fibres originate in the nucleus supraopticus. He leaves the question open whether fibres in this tract also come from the anterior hypothalamic nuclei and from the medial forebrain bundle. A system of more posterior fibres (called tubero-hypophyseal tract) probably originates in the lateral tuberal nuclei and was also followed in the direction of the mamillary bodies and the tegmentum. No fibres were found in the adenohypophysis.

Methods

The following descriptions are mainly based on the material of *Columba livia*, since a sufficient number of good series was present only of this species. The observations from other species are briefly mentioned. It was found that a satisfactory impregnation of the hypothalamic and neurohypophyseal fibres could be obtained by Cajal's block impregnation

method (R § 1804), and still better by Faworsky's modification of the same method (R § 1919). However, these methods never give a perfectly uniform impregnation and this is especially fatal to superficial parts such as the eminentia and the neural lobe. The best impregnation was obtained after treatment of thick (10—20 μ) sections according to Bodian (1936) and Palmgren (1948). Both these methods were equally useful, but the latter is time-saving and does not include usage of protargol, which is difficult to obtain and little reliable as to its properties. The fixation of the material had to be made under deep ether anaesthesia by injection from the carotis if the result should be good, and both horizontal, transversal and sagittal sections had to be used. Bouin did not give as perfect results as the alcohol-formalin-acetic acid mentioned on page 14. The treatment with alcohol had to be short. It should be noted that the coarse fibres of the tractus supraoptico-hypophyseus are often distinct also in less satisfactory preparations whereas the fibres from the tuber region are very delicate and difficult to impregnate.

A very valuable method to demonstrate the neurosecretory fibres of the tractus supraoptico-hypophyseus proved to be the chromic haematoxylin of Gomori (1941, cited by Lillie 1948, p. 96), as was recently shown by Bargmann and Hild (1949) for fish and mammals.

A number of other nerve impregnation methods was tried but found less satisfactory and failed to demonstrate the hypothalamic fibre systems completely, e.g. Golgi methods (R § 1773), Bielschowsky methods (R § 1912, 1909), impregnation according to Gros-Schulze (R § 1793, 1808), Lawrentjew (R § 1915). The methods which require frozen sections failed mainly because the delicate structures of the eminentia and the neural lobe are destroyed during the treatment of the sections, and no continuous series are obtained.

The Innervation of the Neurohypophysis of the Pigeon

The majority of fibres in the neural lobe of the pigeon arrive from the hypothalamic nuclei via the infundibular stem. In addition a very small number of fibres enters the lobe along the inferior hypophyseal artery. These fibres originate in the autonomic nerve plexus around the carotids and are of minor interest partly because they are very few in number and partly because they probably represent a vascular innervation. In the following the hypothalamic part of the innervation is described, and a few notes on the fibres from the carotis are given on p. 232.

Behaviour of the Fibres within the Neurohypophysis

It was mentioned in the preceding chapter that the coarse nerve fibres in the fibre layer of the eminentia mediana and in the stem have a prevailing longitudinal course. The fibres can be followed into the neural lobe, where they form a thick layer near the lumen and finally spread into the peripheral parts of the lobe and form a dense tangle.

When the fibres are followed in detail from the neural lobe towards their origin in hypothalamic centres it can be seen, however, that the pattern is more complicated. At the junction between the neural lobe and the stem all the fibres from the neural lobe are concentrated in a tube-shaped tract which surrounds the lumen of the stem. Practically, no fibres are present outside this tract in the narrow glandular layer or inside it in the ependymal layer. Further rostrally, at the

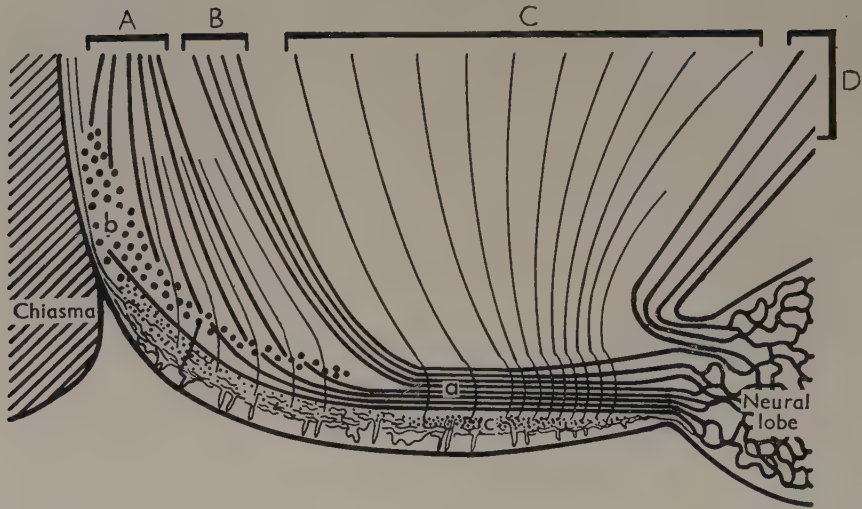


Fig. 121. Diagram showing the behaviour of the nerve fibres in the neurohypophysis of the pigeon (*Columba livia*).

A = tractus hypophyseus anterior, B = tr. supraoptico-hypophyseus, C = tr. tubero-hypophyseus, D = tr. hypophyseus posterior.

a = fibre layer of the eminentia (tr. hypophyseus), b = decussation of the tr. hypophyseus anterior, c = decussation of the superficial eminentia plexus, which is indicated with delicate lines.

junction between the stem and the eminentia, the tube-shaped tract opens dorsally and continues as a mantle in the fibre layer of the eminentia to the region behind the chiasma (fig. 121). The fibre layer thus consists mainly of coarse elements which terminate in the neural lobe. These distinct fibre paths are all included in the term *tractus hypophyseus*. The tract consists, however, of several different portions which will be described in the following.

Just at the base of the stem a few coarse fibres separate from the tractus hypophyseus and turn upwards along the posterior surface of the hypothalamus. This portion is called *tr. hypophyseus posterior* (fig. 121, D).

Outside the fibre layer in the eminentia and in the proximal parts of the stem there is a diffuse plexus of delicate and irregular fibres, which will be called *superficial eminentia plexus*. In addition to numerous fibres with an irregular course this plexus contains a high proportion of transversal fibres, which are concentrated to a constant and distinct decussation near the junction between the eminentia and the stem (fig. 121 c). Transversal fibres are also common more rostrally in the superficial plexus and sometimes form another decussation behind the chiasma. This superficial plexus is practically isolated from the coarse bundles of the fibre layer in the caudal part of the eminentia, but in the rostral part of the eminentia the connection with the tractus hypophyseus is more intimate. The superficial eminentia plexus is responsible for the innervation of the glandular layer, at the base of which it is situated. The nerve fibres in the glandular layer,

including the peculiar loops shown in figures 110 and 113, seem to originate exclusively in this plexus except in the most rostral part of the eminentia as will be mentioned below.

The superficial eminentia plexus is, mainly at least, formed by fibres from the tuber nuclei. The fibres from these nuclei (the *tractus tubero-hypophyseus*) enter the eminentia in two different ways. Some fibres arrive along the lateral surfaces of the hypothalamus and enter the eminentia close to the intima pia outside the tractus hypophyseus. Other fibres from the tuber nuclei pass down to the eminentia along the ventricle and arrive in the ependymal layer inside the tractus. They often run ventro-medially for some distance between the tractus and the ventricle but then suddenly turn outwards, pass between the bundles of the tractus in a direction perpendicular to the surface and join the superficial plexus (fig. 121, 122—124, 126). The fibres radiating through the tractus hypophyseus in this way are very numerous and distinct in the preparations. It can therefore be concluded that the superficial eminentia plexus is formed mainly by fibres from the tuber nuclei, the more so as connections between this plexus and the tractus hypophyseus are rare in the caudal part of the eminentia.

The conditions are more complicated in the rostral third of the eminentia. There is still a superficial plexus, but it is no longer distinct from the mighty fibre layer inside it. It can also be seen that the glandular layer receives some fibres from bundles in the deeper parts of the wall. Some of the peculiar loops in the glandular layer definitely come from the coarse fibres of the tractus hypophyseus. However, also this part of the superficial plexus receives numerous fibres from the nearby tuber nuclei, and the fibres here mainly arrive through the fibre layer (fig. 127).

The rostral part of the eminentia is characterized by the behaviour of the tractus hypophyseus. In a median section and also in transversal sections it can be seen that a large number of its fibres decussate in the region just behind the chiasma. The decussation is situated close to the ependyma and extends from the rostral third of the eminentia upwards behind the chiasma (fig. 121 b). Not all fibres of the tractus hypophyseus decussate here, however, but a distinct and large bundle on each side runs directly upwards along the postoptic slope. These uncrossed fibres, perhaps together with a few crossed ones, form a distinct tract which can be followed to the nucleus supraopticus. It is therefore called *tractus supraoptico-hypophyseus* (fig. 121: B).

The crossed fibres of the tractus hypophyseus can be followed to regions just behind and above the chiasma and also to the praeoptic area. As the exact origin of these fibres is uncertain in many cases I have chosen the neutral term *tractus hypophyseus anterior* for this system. The tr. hypophyseus anterior thus includes all hypophyseal fibres behind the chiasma which do not belong to the tr. supraoptico-hypophyseus or the tr. tubero-hypophyseus (fig. 121: A).

It may be said already here that the Gomori preparations support the above description, which was based on silver preparations. They definitely show that the tr. supraoptico-hypophyseus is mainly destined for the neural lobe, and that

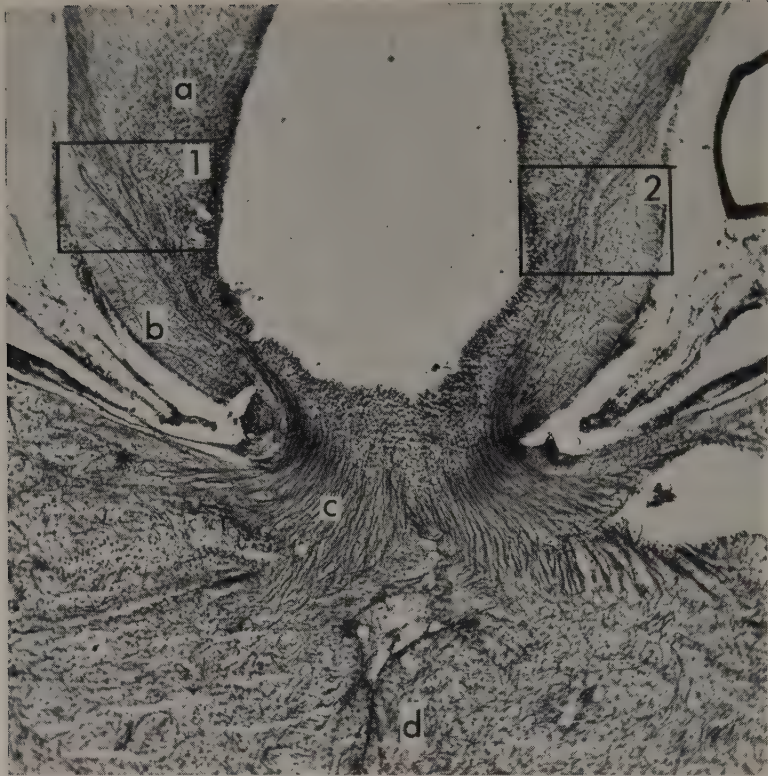


Fig. 122. *Columba livia*, ad. Horizontal section through the neurohypophysis, impregnated according to Palmgren for nerve fibres. a = nucleus tuberis, b = superficial eminentia plexus in the glandular layer of the eminentia, c = tractus hypophyseus, d = lobus nervosus. High-power magnification of the squares 1 and 2 are shown in fig. 123 and 124 resp. — Alcohol-formalin-acetic acid, paraffin 15 μ , impregnation of nerve fibres with silver according to Palmgren. 90 $\times$.

it has little to do with the glandular layer of the eminentia and nothing at all with the caudal part of it (p. 221 ff.). This is in good agreement with the statement above that the glandular layer is supplied mainly by the tr. tubero-hypophyseus.

The Origin of the Nerve Fibres in the Brain

The dense tangle of nerve fibres in the hypothalamus and the absence of discrete tracts make it very difficult to follow the hypophyseal fibres to their origin. In some cases it has been possible to state definitely that a certain nucleus is connected with these fibres, but in other cases only the main direction from which the fibres come could be ascertained. Therefore, I have to leave the question open whether the tractus infundibuli and the medial fore-brain bundle contribute to the hypophyseal innervation and also the rôle of the periventricular nuclei in the anterior hypothalamus is somewhat uncertain.

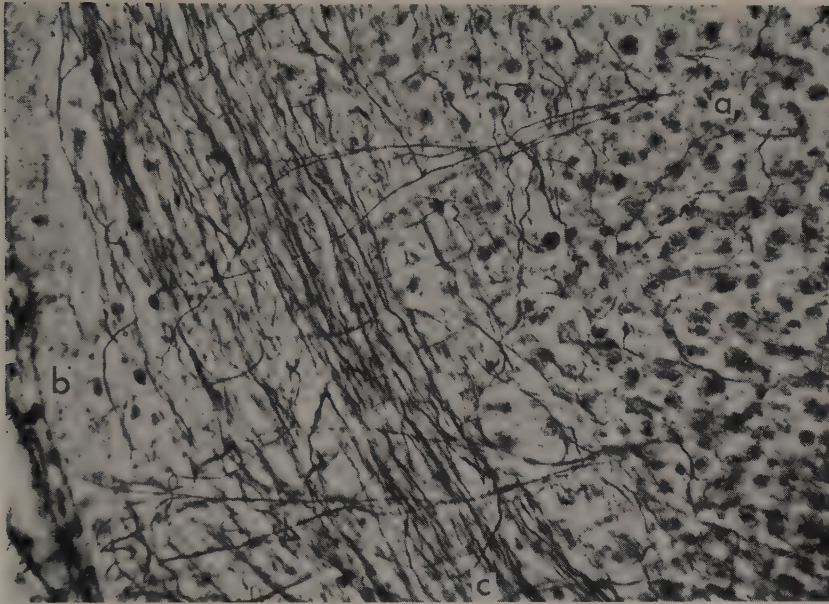


Fig. 123. Square 1 in fig. 122. It can be seen that the fibres from the n. tuberis (a) pass through the tractus hypophyseus (c) and enter the glandular layer (b). 460 $\times$.

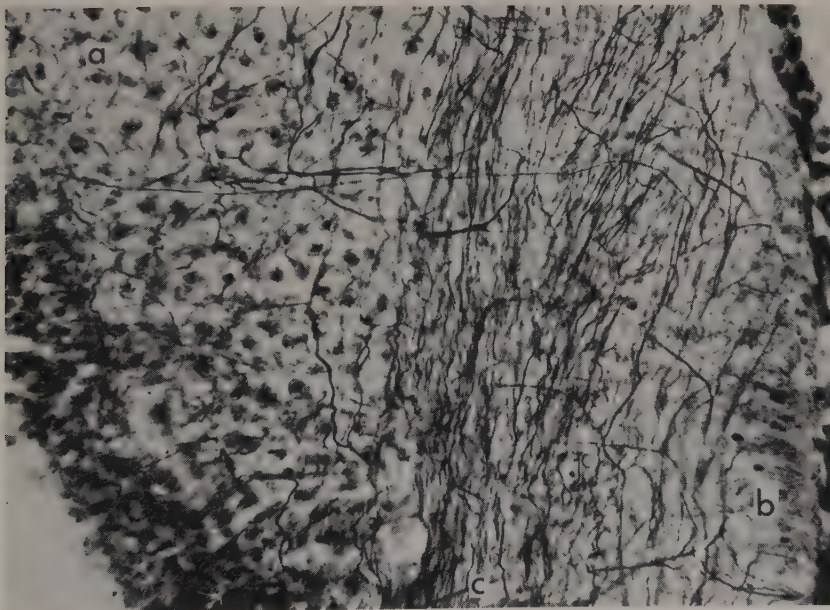


Fig. 124. Square 2 in fig. 122. For explanation cf. fig. 123. — 460 $\times$.

The Tractus hypophyseus posterior

This system consists of fairly few and coarse fibres which leave the neural lobe together with the main hypophyseal tract but separate from it at the very base of the stem. The fibres can be followed upwards along the posterior wall of the hypothalamus approximately to the level of the decussatio tractus infundibuli (Huber and Crosby 1929, = commissura supra-infundibularis, Frey 1937). Some of the fibres arise from scattered large nerve cells situated between the nuclei tuberis and the external wall, but most of them pass on to a diffuse nucleus which is situated just ventrally of the decussatio tractus infundibuli (fig. 125, 126). The nucleus will be called *n. subdecussationis*, but it is so small and indistinct that I hesitate to give it a name. It forms a transversal band of fairly large nerve cells between the decussation and the nucleus mamillaris (Kuhlenbeck 1937, = *n. mam. medialis pars dorsalis*, Huber and Crosby 1929). Only the cells in the ventro-caudal part of the *n. subdecussationis* seem to be connected with the hypophyseal fibres, the other cells send their processes into the tractus infundibuli.

The tr. hypophyseus posterior is not distinct in all pigeons in my material. It is much better developed in my specimens of *Anser* and also much easier to distinguish from the tr. tubero-hypophyseus in this species due to the striking difference in the thickness of the fibres.

The nucleus mamillaris (Kuhlenbeck 1937, = *n. mam. medialis dorsalis* of Huber and Crosby 1929) does not contribute to the hypophyseal innervation as far as evidenced by my Bodian and Palmgren preparations.

The Tractus tubero-hypophyseus

The fibres which constitute the tr. tubero-hypophyseus originate in the region immediately dorso-laterally of the eminentia. This most ventral portion of the hypothalamus contains in the pigeon, as in other birds, a uniform mass of small and dense cells on each side of the ventricle: the nucleus tuberis (Kuhlenbeck 1937, = *n. hypothalamicus inferior* + *n. mamillaris medialis ventralis*, Huber and Crosby 1929). The nucleus tuberis is probably identical with the "nucleus m" of Rendahl (1924). It is not perfectly delimited from the *n. mamillaris*. The two nuclear masses, which both extend along the ventricle, are continuous, but there is a distinct constriction of the nuclear layer between them (fig. 125, 126). The nuclei tuberis become narrower more rostrally, but extend also for some distance upwards along the posterior slope of the chiasma.

In transversal sections (fig. 126) it can be distinctly seen that the fibres of the tr. tubero-hypophyseus come from the nuclei tuberis and do not pass further dorsally beyond the limit of these nuclei to the nuclei mamillares. The fibres from the tuber cells behave in two different ways as described above. Most of them pass straight laterally to the surface and then follow it to the superficial plexus of the eminentia, and some run along the ventricle downwards and have to penetrate

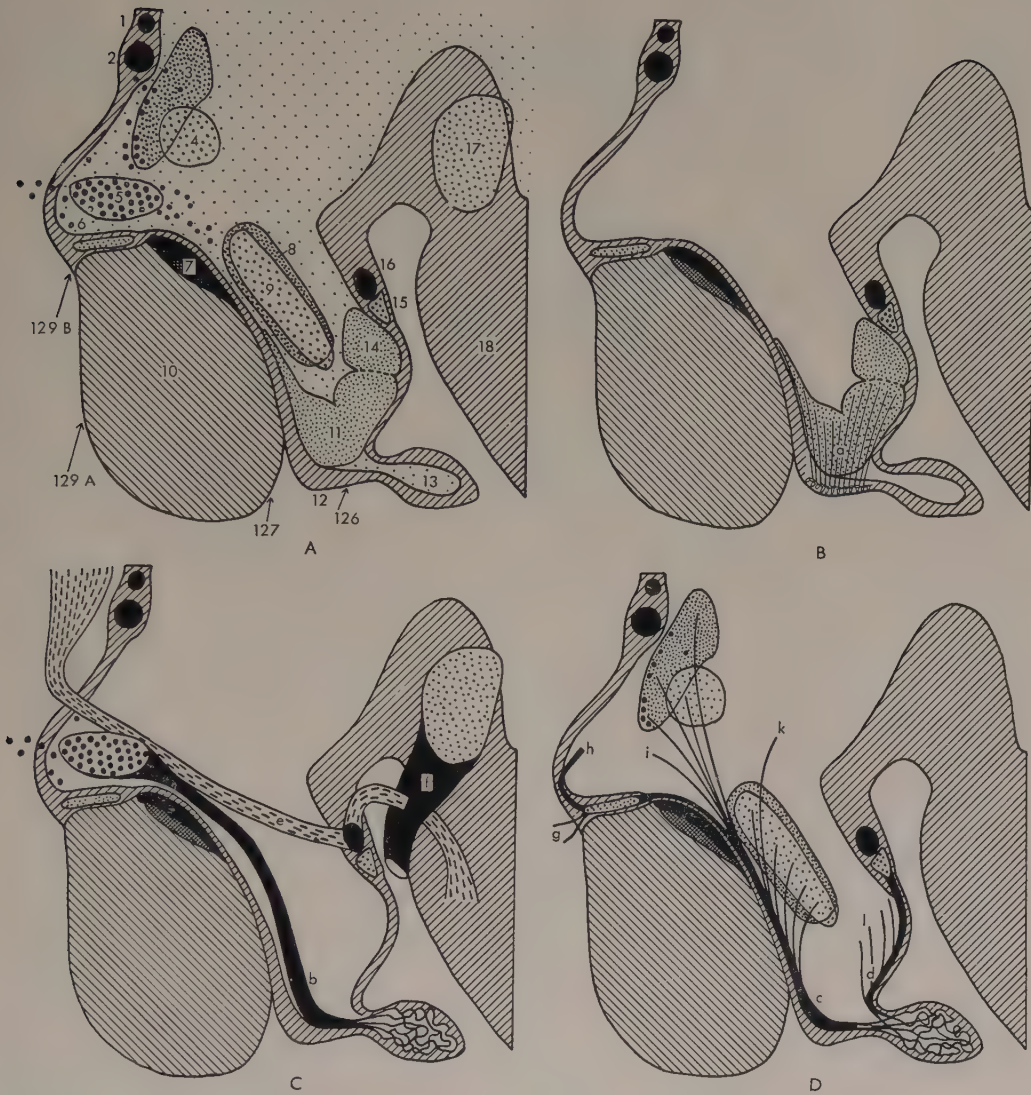


Fig. 125. Diagrams showing the innervation of the avian neurohypophysis. Fig. A shows the situation of the nuclei, fig. B the tractus tubero-hypophyseus, fig. C the tr. supraoptico-hypophyseus, fig. D the tr. hypophyseus anterior and posterior.

Explanation to fig. A: 1 = commissura pallii, 2 = commissura anterior, 3 = principal and 4 accessory part of the n. paraventricularis magnocellularis, 5 = n. supraopticus, 6 = recessus praeopticus with the median praeoptic nucleus in its ventral wall, 7 = supraoptic decussations, 8 = n. inferior hypothalami, 9 = n. lateralis hypothalami, 10 = chiasma, 11 = n. tuberis, 12 = eminentia mediana, 13 = neural lobe, 14 = n. mamillaris, 15 = n. subdecussationis, 16 = decussatio tractus infundibuli, 17 = nuclei of nervus oculomotorius (simplified), 18 = medulla oblongata. The arrows indicate the planes of figures 126, 127 and 129.

Explanations to fig. B—D: a = tr. tubero-hypophyseus, b = tr. supraoptico-hypophyseus, c = tr. hypophyseus anterior, d = tr. hyp. posterior, e = tr. infundibuli, f = nervus oculomotorius, g = autonomic fibres from fasciculus opticus, h = "fasciculus supraopticus" (Rhötig), i = fibres, probably from praeoptic areas, k = fibres, probably from paraventricular areas, l = fibres from cells near the surface of the posterior hypothalamus.

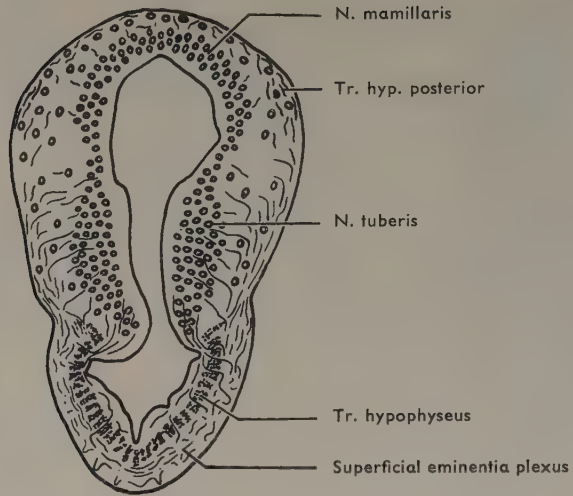


Fig. 126. *Columba livia*, ad. Cross section through the posterior part of the hypothalamus, corresponding to the plane 126 in fig. 125 A. — Alcohol-formalin-acetic acid, paraffin 15μ , impregnation with silver according to Palmgren. $35\times$.

the fibre layer before they reach the superficial plexus. It could not be stated that fibres from the tuber nuclei join the main tractus hypophyseus. Though it is possible that this occurs — the dense tangle of fibres does not allow a definite statement — the main terminus of the tractus tubero-hypophyseus is undoubtedly the glandular zone of the eminentia. As decussating fibres are very numerous in the superficial eminentia plexus it must be concluded that the majority of the fibres from the tuber nuclei cross over to the other side here.

The nuclei tuberis, like the n. mamillaris, are connected with a large number of non-medullated fibres which descend from the praeoptic region.

The Tractus supraoptico-hypophyseus

The fibres of this tract separate from the median portion of the tractus hypophyseus in the rostral part of the eminentia and can be followed in a rostro-lateral direction upwards along the posterior slope of the chiasma. They keep together fairly well and can therefore easily be followed up to the level of the supraoptic decussations. Here the tr. supraoptico-hypophyseus enters into intimate contact with the tr. infundibuli (Huber and Crosby 1929) and runs along the latter tract through the portions of the dorsal supraoptic decussation into the praeoptic area (fig. 125: C, 127). Since the tr. supraoptico-hypophyseus becomes more diffuse when it begins to cross through the lateral parts of the dorsal supra-optic decussation its fibres are here easily confused with the other hypothalamic fibres which have the same direction (e. g. praeoptic-tuberal and praeoptic-mamillary tracts). In some sagittal sections it is possible, however, to follow some bundles of the tr. supraoptico-hypophyseus all the way through the decussating systems

into the praeoptic area where they originate in the large cells of the nucleus supraopticus (Kuhlenbeck 1937), perhaps also in other cells of the lateral praeoptic region. That the course of the tractus is as described here is confirmed also by the Gomori preparations, in which its way is marked by granules of the specific, bluish-staining colloid (p. 222—223).

The nucleus supraopticus will be described more in detail below because of its neurosecretory activities. It extends above the tractus opticus from the neighbourhood of the ventro-lateral angle of the praeoptic recess latero-caudally into the lateral parts of the dorsal supraoptic decussation (fig. 125, 129). Scattered neurosecretory cells of the same type are found along the tractus also behind the decussations in some specimens.

Green (1951) mentions the possibility that also fibres from the septal and cortical areas may enter the pituitary. As described above the tr. supraoptico-hypophyseus has a very close relation to the tr. infundibuli and also to the septo-mesencephalic tract which is situated just dorsally of the infundibular tract in the posterior hypothalamic area. In my preparations the tr. infundibuli could be followed to the nuclei immediately in front of the anterior commissure (fig. 125 C). It is possible that fibres from these tracts join the tr. supraoptico-hypophyseus and that direct connections between septal areas and pituitary thus exist. It is also possible that descending fibres from cortical and septal areas join the bundles of the tr. hypophyseus anterior, but this could not be stated with certainty.

The Tractus hypophyseus anterior

It was said above that the term tr. hypophyseus anterior is used tentatively for the large number of fibres which descend the post-optic slope and enter the tractus hypophyseus, with the exception of the tr. supraoptico-hypophyseus which was dealt with above. The tr. hyp. anterior forms a diffuse system of fibres in more medial parts of the postoptic hypothalamus between the two tr. supraoptico-hypophyseus (fig. 125 D, 127). Most of the fibres seem to cross in the postoptic decussation. The fibres arrive from very different nuclei and it proved very difficult to state their origin in some cases, because they become mixed with fibres of other systems.

It is easily seen that a great number of the fibres in this tract comes from the n. lateralis hypothalami (Kuhlenbeck 1937). The more medially situated n. inferior hypothalami (Kuhlenbeck) emits fairly few, if any, hypophyseal fibres (fig. 127).

A mighty but diffuse system of fibres from the tr. hypophyseus anterior has a more medial course and arrives from directions corresponding to Kuhlenbeck's n. paraventricularis posterior hypothalami, n. paraventricularis magnocellularis and n. praeopticus paraventricularis. The silver preparations do not show exactly which of these nuclei give rise to the hypophyseal fibres, but the Gomori preparations indicate that periventricular cells ventrally of the anterior commissure are connected with the pituitary (p. 224—225) (fig. 125: D, 127).

Finally, it can be distinctly seen that a number of more median fibres from the tr. hypophyseus anterior run upwards along the posterior slope of the chiasma, pass over the supraoptic decussations and enter the praeoptic region. Some of these fibres may, however, synapse in the nerve cells behind the chiasma, e. g. in the n. inferior hypothalami.

Of certain interest from a functional point of view are connections between the optic fibre systems and the pituitary. Frey (1937) described an unpaired hypothalamic root of the opticus in *Cavia* and *Homo* and later (1938 and 1938 a) in different Amphibia. The partly non-medullated fibres of this root are said to come from the dorsal part of the chiasma, and some of them are direct, i. e. they do not cross in the chiasma. The hypothalamic root is said to terminate in ganglion cells situated in the wall of the recessus praeopticus. In *Cavia* this ganglion protrudes into the praeoptic recess as a median ridge. Berthoud (1943) also mentions direct hypothalamic fibres from the opticus in his embryological investigation of the optic roots in *Gallus*. He says, however, that the hypothalamic fibres terminate in the nucleus ovoideus and he can therefore hardly have seen the same fibres as Frey.

Frey's statements have later been criticized by Bodian (1940) in a paper on the diencephalon of the opossum. Bodian suggests that the "hypothalamic optic root" in fact is the fasciculus supraopticus of Rhötig (1909), partly also some ordinary optic fibres which have been misinterpreted.

It is unquestionable that fibres from the fasciculi optici enter the praeoptic area in birds. They were definitely observed in *Columba* and *Anser*, the only species of which silver preparations of the entire praeoptic area were available. However, the fibres are very few and it is far from certain that they arrive from the retina.

In pigeons the bottom of the praeoptic recess is in contact with the chiasma and presents a high median ridge which extends from the top of the recess to the neighbourhood of the dorsal supraoptic decussation. This ridge contains a rich system of non-medullated and delicate nerve fibres which run in a longitudinal direction. It also contains a moderate number of small, spindle-shaped nerve cells which are orientated along the fibres (fig. 125, 129). The ridge corresponds well to the ganglion of the hypothalamic root described by Frey (1937) in *Cavia*.

At the point of the praeoptic recess the fibres enter the median ridge from two directions. Some of them descend from the anterior (dorsal) wall of the praeoptic recess and arrive at the end of the recess as two more or less distinct bundles, one on each side. These fibres seem to originate in the region dorso-laterally of the praeoptic recess and have a course, approximately corresponding to the "fasciculus supraopticus" of Rhötig (1909) (cf. fig. 125 D).

A minor portion of the fibres in the praeoptic ridge can be followed along the dorsal surface of the chiasma *far rostrally of the point of the praeoptic recess*. Near the end of the recess they still form a more or less diffuse median bundle, but further rostrally they spread to the dorsal surface of the fasciculi optici. The fibres are much thinner than those of the common optic bundles and can therefore be recognized even when they become mixed with the optic fibres. Frey (1937) is

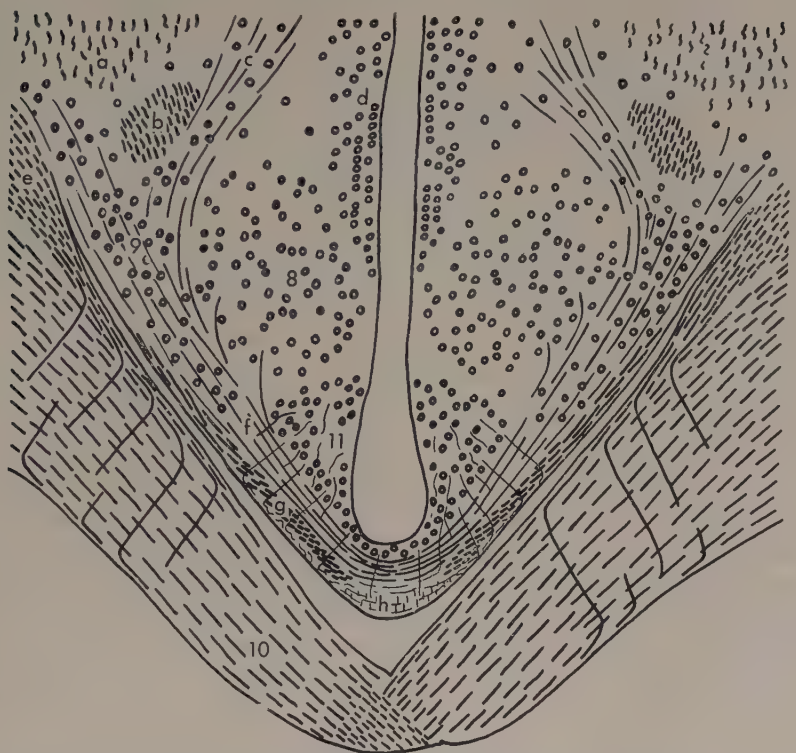


Fig. 127. *Columba livia*, ad. Transversal section through the rostral part of the eminentia (the plane is indicated in fig. 125 A).

8 = n. inferior hypothalami, 9 = n. lateralis hypothalami, 10 = chiasma, 11 = n. tuberis.

a = ramus basalis caudalis of tr. septo-mesencephalicus, b = tr. infundibuli, c = medial fibres of tr. hypoph. anterior, probably from periventricular thalamic areas, d = n. paraventricularis posterior, e = tr. opticus basalis (Frey 1937), f = tr. hypoph. anterior, g = tr. supraoptico-hypophyseus, h = superficial eminentia plexus.

Camera lucida drawing. Fibres and cells are much more numerous in the original. — Alcohol-formalin-acetic acid, paraffin 15 μ , silver impregnation according to Palmgren. 35 $\times$.

of the opinion that the hypothalamic optic fibres in the mammals are autonomic and the morphological appearance of these fibres in birds speaks in favour of a similar interpretation.

It should be pointed out, however, that the fibres which constitute the "hypothalamic optic root" are very few in the pigeon. Their exact number is difficult to state for they are rarely perfectly impregnated, but it is certainly less than a hundred. Further, they have not been followed far rostrally since the preparations do not include more than the very base of the fasciculi optici. It is thus not ascertained that they really arrive from the retina. When passing along the surface of the opticus these fibres are regularly accompanied by numerous glia cells which form islands of glious tissue on the surface of the large optic bundles. It is thus

open to some doubt, whether these "hypothalamic optic fibres" can transfer optic stimuli to the hypothalamus.

On the other hand, if they are able to transfer optic stimuli to the median ridge of the praeoptic recess, this means that an almost direct connection between the eye and the pituitary exists. If the fibres of the median ridge are followed caudally it can be observed that the ridge becomes lower and finally disappears near the dorsal supraoptic decussation. Most fibres maintain their straight caudal course, however, and spread as a narrow and flat layer on the surface of the decussation. Further caudally they dive down to the eminentia and join the fibres of the tr. hypophyseus anterior, still closely attached to the posterior surface of the decussation and the chiasma (fig. 125 D). On their way from the praeoptic region to the eminentia these fibres are accompanied by praeoptic ependymal fibres which encircle the chiasma and attach to the brain surface in the furrow between the chiasma and the eminentia.

Notes on the Innervation of the Neurohypophysis in Other Birds

The behaviour of the nerve fibres in the neurohypophysis, especially the eminentia mediana, has been studied in a considerable number of birds which are represented by Bodian preparations in my collection (cf. table 1). It could be stated that the pattern of the eminentia described above for the pigeon is constant as regards the essential features. There is always a superficial net of finer fibres at the base of the glandular layer. This superficial net contains several transversal fibres which are concentrated to a distinct decussation near the attachment of the stem, sometimes also to a less distinct decussation near the chiasma. The superficial eminentia net seems always to be restricted to the eminentia and does not reach beyond the base of the stem caudally. The tractus hypophyseus has a longitudinal course through the eminentia and invariably forms a distinct decussation just behind the chiasma as in the pigeon.

A study of the origin of the nerve fibres in the brain requires continuous and well impregnated Bodian or Palmgren preparations of the entire hypothalamus and pituitary. Such series are present of *Anser anser* and *Anas platyrhynchos* and a good though somewhat incomplete series of *Branta leucopsis* also proved valuable. The results obtained from the pigeon material were in general corroborated by this material, and the differences mainly concern shape etc.

The tractus hypophyseus posterior is especially well developed in the preparations of *Anser*. The fibres are here more numerous than in *Columba* and can easily be distinguished from the tuber fibres owing to the striking difference in thickness. The majority of the fibres of the posterior hypophyseal tract arrive from the lower part of the nucleus subdecussationis (cf. p. 214). Most fibres from this nucleus pass laterally to the external surface before they turn ventrally to the base of the stem, and the tract thus tends to be paired.

The tractus tubero-hypophyseus is probably also in these species the source of

the superficial eminentia net. The nuclei tuberis emit numerous fibres in the direction of the neurohypophysis, but the plane of the sections did not permit a detailed study of the course of these fibres within the eminentia.

The tractus supraoptico-hypophyseus is very distinct in all the specimens. As in the pigeon it separates from the decussating fibres of the tractus hypophyseus and runs laterally of most other hypophyseal fibres in the postoptic region. It could be distinctly followed along the tractus infundibuli through the supraoptic decussation into the praeoptic region. In the goose and the duck it could even be definitely stated that these fibres come from the large neurosecretory cells of the nucleus supraopticus.

The remainder of the fibres of the hypophyseal tract, collected under the term tractus hypophyseus anterior, come from areas dorsally and dorso-caudally of the chiasma and also from the praeoptic region.

The median praeoptic nucleus is distinct as a longitudinal ridge in *Branta* and *Anser* but had been damaged in the preparations of *Anas*. In the two first-mentioned species it receives some fibres from the dorsal surface of the chiasma and the fasciculi optici, but it receives mighty bundles of non-medullated fibres from the rostral surface of the praeoptic recess. Caudally some fibres of the median praeoptic ridge can be clearly followed over the dorsal supraoptic decussation down to the eminentia.

Neurosecretion and Pituitary in the Pigeon

Bargmann (1949) and Bargmann and Hild (1949) have shown that the neurosecretory neurons in the hypothalamic area including the axons can be selectively stained with the chromic haematoxylin of Gomori. They used dog, cat and tench (*Tinca tinca*) as experimental animals, and in all cases they obtained very good results. The substance stained specifically with the haematoxylin is regarded as a secretory product, formed by the ganglion cells of the nucleus supraopticus and paraventricularis (resp. n. praeopticus in *Tinca*), and is transported to the neurohypophysis along the axones of the cells. Drager (1950) made such a transport of hypothalamic colloid to the neurohypophysis still more probable by experiments with the snake *Spilotes corais*. After hypophysectomy the sella of the snake was filled with fibrin foam. Some weeks after the operation the foam contained numerous granules of the same specific kind as the matter produced by the supraoptic nucleus. Later Ortmann (1951) has described in detail the distribution of the Gomori-positive matter in the hypothalamus of the rat under normal and experimental conditions. He concludes that the substance is functionally related to the diuretic principle of the neurohypophysis, either being this principle itself or its carrier.

I have used Gomori staining for a number of reptilian and avian specimens. Beautiful results were obtained in *Tropidonotus natrix*, but in other reptiles the staining of the supraoptic neurons was less intensive, and the same is true also

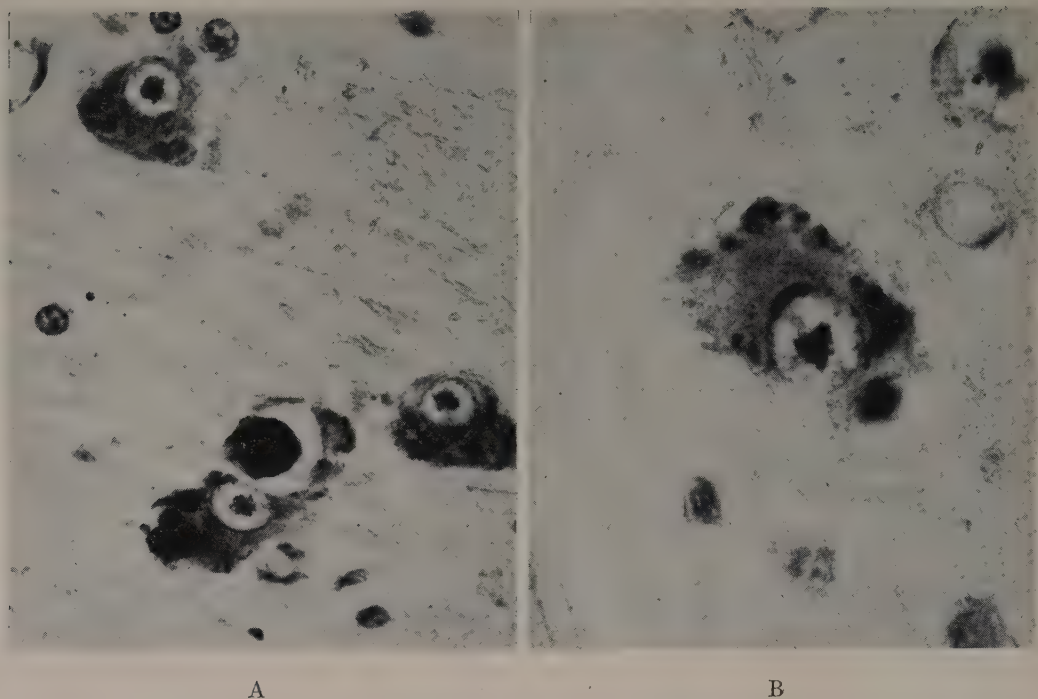


Fig. 128. *Columba livia*, ad. Neurosecretory cells in the n. supraopticus. In A some cells with large black bodies of Gomori-positive substance, in B the "resting-stage". — Bouin, paraffin 6 μ , Gomori's haematoxylin and phloxin. A 800 $\times$, B 1500 $\times$.

for most birds, although the colloid of the neural lobe was always perfectly demonstrated.

Three series of pigeons were stained according to the Gomori technique and show approximately the same. The neural lobe of the hypophysis is more or less completely filled with the granular, bluish-black Gomori-substance, which tends to aggregate in the "glandular zones" along its surface and around the vessels. The tractus hypophyseus in the fibre layer of the stem and of the eminentia also contains granules of this substance, and many fibres here are blue in contrast to other nerve fibres which are red by the phloxin used as counterstain. The glandular layer of the eminentia is practically free from Gomori-substance in its caudal parts, but just behind the chiasma also the glandular layer contains scattered Gomori-positive granules (cf. fig. 130).

The bluish fibres with accompanying granules of the blue-black Gomori-substance can be traced further rostrally and appear as a rather discrete tract (tr. supra-optico-hypophyseus) on each side upwards along the posterior slope of the chiasma. When the contact with the tractus infundibuli is established the staining of the fibres is but slight, however, and the further course of the tract can be followed only due to the presence of scattered blue granules in the tissue. These granules

are numerous along the course of the tr. supraoptico-hypophyseus in one of the specimens, through the lateral parts of the dorsal supraoptic decussation into the praeoptic region where the tract reaches its origin in the nucleus supraopticus and in scattered neurosecretory cells in the surroundings. Though neurosecretory fibres can be followed also in a more medial and caudal direction from the anterior eminentia (in the tr. hypophyseus anterior) it is not possible to follow them to their probable origin: the scattered neurosecretory cells in the anterior part of the paraventricular nucleus. Scattered Gomori-positive granules were found in the neighbourhood of this nucleus, but they are too rare to mark out the tract distinctly.

The neurosecretory ganglion cells in the pigeon have essentially the same appearance as described by Bargmann for mammals. They contain a bluish grey or blue-black substance of a fine granular appearance not seen in other diencephalic cells (fig. 128). The plasma is rich and its peripheral parts contain dark bodies, interpreted by Bargmann as dislocated Nissl bodies. Vacuoles of Gomori-positive substance are also common in the cells, but are usually small. In one of the pigeons a relatively large proportion of the neurosecretory cells contains one or more large vacuoles with a blackish content, causing a marked distortion of the cell body. Undoubtedly, the different appearance of the neurosecretory cells indicates differences in the physiological state of the animals. Ortmann's (1951) and Hillarp's (1949) experiments with rats show that the appearance of these cells changes with differences in the water balance of the animal.

The distribution of the neurosecretory cells in the hypothalamus of the pigeon should also be discussed briefly. The majority of cells producing the Gomori-positive substance are present in a nucleus which I call nucleus supraopticus, for reasons mentioned below. It is situated in the praeoptic area laterally of the praeoptic recess and extends latero-caudally along the dorsal surface of the tractus opticus (fig. 125, 129). Scattered cells occur further back along the tractus supraoptico-hypophyseus through the lateral parts of the supraoptic decussation into the anterior part of the post-optic area. The nucleus supraopticus is not very distinctly delimited and its cells tend to spread in different directions. Some parts of it contain exclusively neurosecretory cells of the magnocellular type whereas other parts also contain smaller, non-secretory cells.

The nucleus supraopticus of the present paper is probably identical with the equally termed nucleus in Kühlenbeck's paper on the chick diencephalon (1937) and in Kurotsu's (1935) paper on the nucleus magnocellularis periventricularis in different reptiles and birds. It is probably not identical with the "nucleus supraopticus" described by Huber and Crosby (1929) in the sparrow, but may correspond to parts of the nucleus magnocellularis interstitialis of these authors. Craigie (1931) did not find any nucleus in the humming-bird to which the name nucleus supraopticus could be given. In its general distribution the nucleus supraopticus in the pigeon corresponds to the equally named nucleus in reptiles (Kurotsu 1935, Meyer 1935 etc.) and in mammals (e. g. Meyer 1935, Boon 1938, Ortmann 1951).

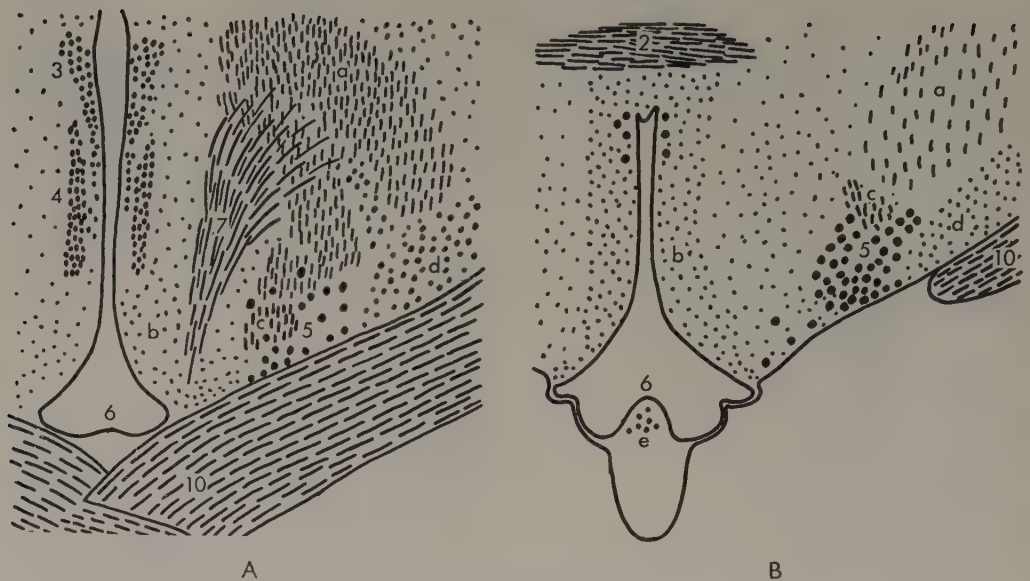


Fig. 129. *Columba livia*, ad. Cross sections through the praeoptic region. A immediately in front of the supraoptic decussations, B through the commissura anterior. The planes are indicated in fig. 125 A.

2 = commissura anterior, 3 = principal and 4 = accessory part of the nucleus paraventricularis magnocellularis, 5 = n. supraopticus, 6 = recessus praeopticus, 7 = decussatio supraoptica dorsalis, 10 = chiasma and tractus opticus.

a = forebrain bundles, b = praeoptic periventricular nuclear masses, c = tractus infundibuli, d = nucleus, corresponding to the n. ventrolateralis in the chick (Kuhlenbeck), e = median praeoptic nucleus.

Camera lucida drawings of $10\ \mu$ sections, fixed in Bouin and stained in Gomori's haematoxylin and phloxin. Nerve tracts only approximately indicated. $35\times$.

Also other parts of the praeoptic area may contain scattered neurosecretory cells, from the region just below the anterior commissure to the chiasma, but they are rare and do not form distinct nuclei. It is curious to note that also the ventromedial parts of the hemispheres contain scattered neurosecretory cells far in front of the level where the praeoptic recess disappears in a series of transversal sections. This supports the view that also forebrain fibres enter the pituitary.

The second region where a concentration of neurosecretory cells is distinct is the nucleus paraventricularis. Kurotsu (1935) who described carefully this nucleus in the pigeon and some other birds, recognized two portions of the n. paraventricularis magnocellularis: the principal nucleus ("Hauptkern") and the accessory nucleus ("Nebenkern"). Both nuclei extend along the ventricle in the region behind the praeoptic area and are connected with each other (fig. 125, 129). In my preparations neurosecretory cells are common in the periventricular cell layer near the praeoptic area, but it is uncertain whether these cells should be referred to the nucleus praeopticus dorsocaudalis (Kurotsu, Kuhlenbeck) or to the principal part of the n. paraventricularis magnocellularis (Kurotsu). The latter nucleus con-

tains very few neurosecretory cells in its more caudal part, and the accessory part of the n. paraventricularis magnocellularis does not show any signs of neurosecretion whatever.

Finally, it should be pointed out, that the above description of the neurosecretory activity in the hypothalamus of the pigeon only concerns the production of Gomori-positive, hypophyseal colloid. It is of course possible that other kinds of neurosecretion may take place in the cells which were regarded as non-secretory above.

The Neurosecretion in Some Other Birds

Two series of sections, one of *Sylvia atricapilla* and one of *Regulus regulus* were stained in the chromic haematoxylin of Gomori. They corroborate perfectly the observations from pigeon material as regards the distribution of the Gomori-positive substance in the neurohypophysis. The neural lobe is packed with the blue-black matter, but the eminentia contains such substance only in the tractus hypophyseus and in the rostral part of the glandular layer, whereas the caudal part of the glandular layer is unstained by the haematoxylin (fig. 130, 131).

The distribution of the neurosecretory cells is, however, somewhat different from that described for the pigeon. The nucleus magnocellularis paraventricularis does not show any signs of secretion in the two specimens. In *Sylvia atricapilla* most neurosecretory cells are found in the nucleus supraopticus, which in this species has spread in different directions. Its main part is situated above and partly between the fibres of the tractus opticus approximately at the place where tr. infundibuli and tr. septo-mesencephalicus cross the optic tract. In addition, there is a large and flat aggregate of intensively secreting cells attached to the surface of the brain just rostrally of this point. This aggregate is believed to correspond to Kurotsu's nucleus magnocellularis praeopticus pars lateralis. In addition, the pars medialis (Kurotsu) contains scattered neurosecretory cells. The Gomori-positive cells of the nucleus supraopticus extend far caudally in two directions. One string of such cells is present along the tr. infundibuli and tr. septo-mesencephalicus and reaches far into the post-optic area. Another string extends ventro-laterally through the dorsal supraoptic decussation and partly between this decussation and the chiasma almost to the inner pole of the nc. ectomamillaris (Huber and Crosby 1929), where a real nucleus of secretory cells is formed.

In *Regulus regulus* there are also few (20—30) secretory cells in the periventricular praeoptic nuclei, and the majority of them are concentrated in the nc. supraopticus, the main part of which has the same relation to the optic tract and to the tr. septo-mesencephalicus and infundibuli as in *Sylvia*. The activity of the supraoptic nucleus could be observed also in four other specimens of *Regulus*, stained in azan or phosphotungstic acid haematoxylin according to Mallory. Scattered groups of neurosecretory cells extend also caudally of the nc. supraopticus along the ramus basalis caudalis of the septo-mesencephalic tract, but they are not continuous with the nc. supraopticus as in *Sylvia*. In addition, there is

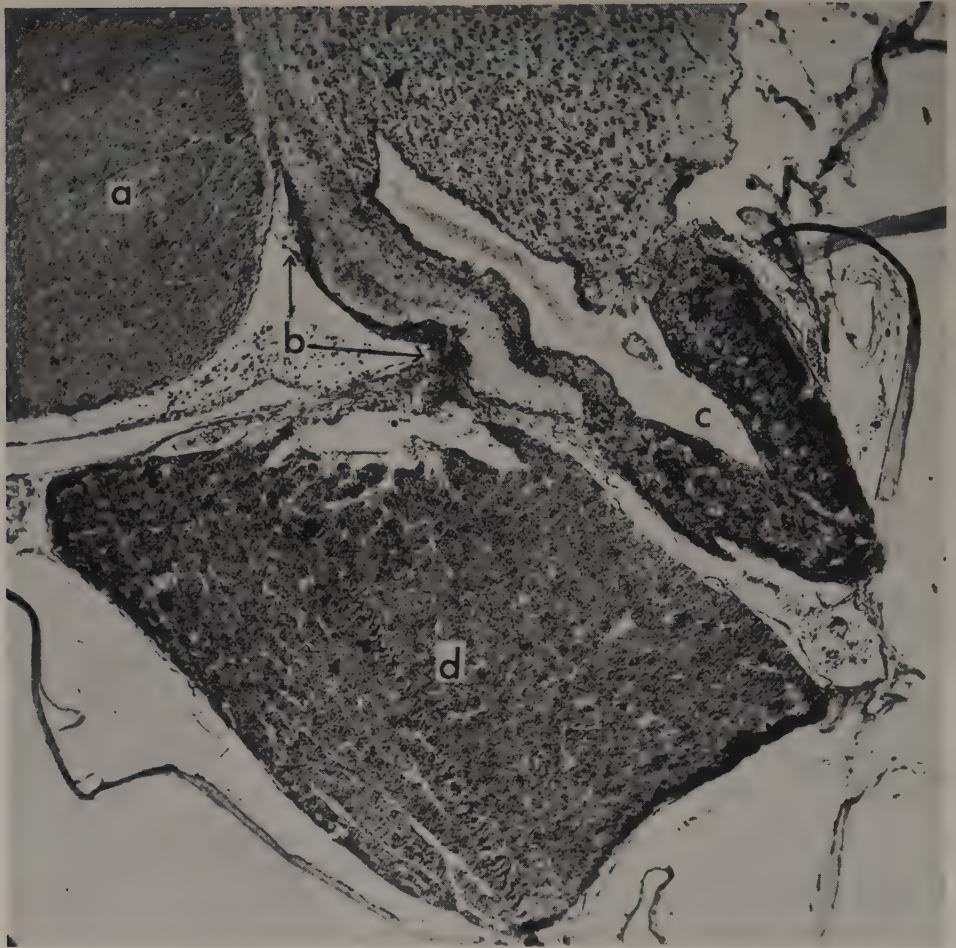


Fig. 130. *Sylvia atricapilla*, ♀ ad. Median section of the pituitary. a = chiasma, b = eminentia mediana, c = lobus nervosus, d = pars distalis. The blackish Gomori-substance is accumulated in the neural lobe and the tractus hypophyseus. The glandular layer of the eminentia contains a few granules in the rostral parts. Bouin, paraffin 8 μ , chromic haematoxylin of Gomori and phloxin. 103 $\times$.

also, in *Regulus*, a fairly great number of secretory cells along the ramus dorsalis of the same tract until it reaches laterally of the nucleus rotundus. This latter extension of the neurosecretory region was observed only in this species.

The Gomori-positive vacuoles in the neurosecretory cells are also distinct in the celloidin sections stained in Mallory's phosphotungstic acid haematoxylin although the sections are 40–100 μ thick. The vacuoles are concentrated in the nucleus supraopticus in the species in which they have been observed, and only few cells are scattered in the praeoptic region and along the septo-mesencephalic and infundibular tracts. A few examples are mentioned here:



Fig. 131. *Regulus regulus*, ♀ ad. Horizontal section through the chiasma (a), the eminentia mediana (b), the lobus nervosus (c) and the oculomotorius (d). The distribution of the (blackish) Gomori-positive matter is the same as in fig. 130. — Bouin, paraffin 8 μ , chromic haematoxylin of Gomori — phloxin. 94 $\times$.

Caprimulgus europaeus. Staining phosphotungstic acid haematoxylin. A few neurosecretory vacuoles are present in the periventricular praeoptic region. The nc. supraopticus is packed with such vacuoles, which also extend caudally along the septo-mesencephalic tract.

Diomedea epomophora. Stained in phosphotungstic acid haematoxylin. The vacuoles are extremely frequent in the nucleus supraopticus, which is situated in the region where the septo-mesencephalic and optic tracts cross. A string of vacuoles extends along the septo-mesencephalic tract caudally to the level of the nucleus ectomamillaris. A number of vacuoles are also present in scattered cells around the praeoptic recess.

The neurosecretory activity connected with the pituitary in the investigated birds may thus be characterized in the following way: The neurosecretory cells are concentrated in the nucleus supraopticus, the greater part of which is situated laterally of the praeoptic recess dorsally of the chiasma at the point where the infundibular and septo-mesencephalic tracts enter the praeoptic area. Scattered secretory cells tend to spread from this nucleus caudally along the septo-mesen-

cephalic tract and also (especially in *Sylvia*) ventro-laterally among the bundles of the dorsal supraoptic decussation. Scattered cells or small groups are also regularly present in the periventricular praeoptic region and, in the pigeon, in the n. paraventricularis magnocellularis. The Gomori-positive substance can be more or less perfectly traced along the tractus supraoptico-hypophyseus to the neurohypophysis where it accumulates in the neural lobe. The glandular layer of the eminentia is free from Gomori-substance in the caudal part and contains only little of such substance in the rostral part. The evidence gained from the silver preparations that the tr. supraoptico-hypophyseus is mainly destined for the neural lobe, and that it has little to do with the glandular layer of the eminentia is thus confirmed by the Gomori technique.

Nerve Endings and Related Structures

The nerve terminals in the neural lobe of mammals have aroused considerable interest (cf. e. g. Romeis 1940, Vasquez-Lopez 1942, Hanström 1946, 1946 a, 1947, 1948, 1950, Bargmann 1949, Collin and Stutinsky 1949, Stutinsky 1949). They have, as far as witnessed by the literature, never been observed in birds.

First it should be pointed out that varicose swellings are fairly common on the fibres in the avian neurohypophysis. Especially the delicate loops in the glandular layer of the eminentia and the fibres in the neighbourhood of the ependyma show a distinct tendency to form thickenings, but swellings on the fibres are also often seen in the neural lobe. In many cases these varicose swellings give the impression of true nerve endings and they may reach the size of a cell nucleus or more (fig. 132: A and B), but the continuation of the fibre can often be seen on the other side. They are very distinct in Bodian preparations and appear as faintly blue "Herring bodies" in azan preparations. More complicated structures in the shape of small nets etc. may also be seen. These structures, though perhaps sometimes prospective "nerve endings", do not deserve this name as the fibre continues on the other side.

True nerve endings are not present in all avian pituitaries as far as demonstrated in Bodian preparations. Thus I have searched for them in vain in most of the pigeon preparations, and also in Bodian slides of *Apus*. They are, however, common in the neural lobe and adjacent parts of the stem in *Anser*, *Branta*, *Phalacrocorax*, *Coragyps* and *Diomedea*, less frequent but still present in the neural lobe of two pigeons (*Columba*), in *Larus argentatus*, *Pica* and *Nycticorax*. Especially the goose is very suitable for studying these nerve endings which occur in a high frequency and show a rich morphological variation in this species.

The most typical shape of these nerve endings is that of a sphaerule or a bulb, stained black or dark reddish in Bodian preparations and surrounded by a lighter sphere, containing a granular substance which stains bluish in azan. This mantle makes it possible to state that only one nerve fibre enters the black sphaerule in most cases. It may be noted that the colloidal mantle attaches firmly and belongs

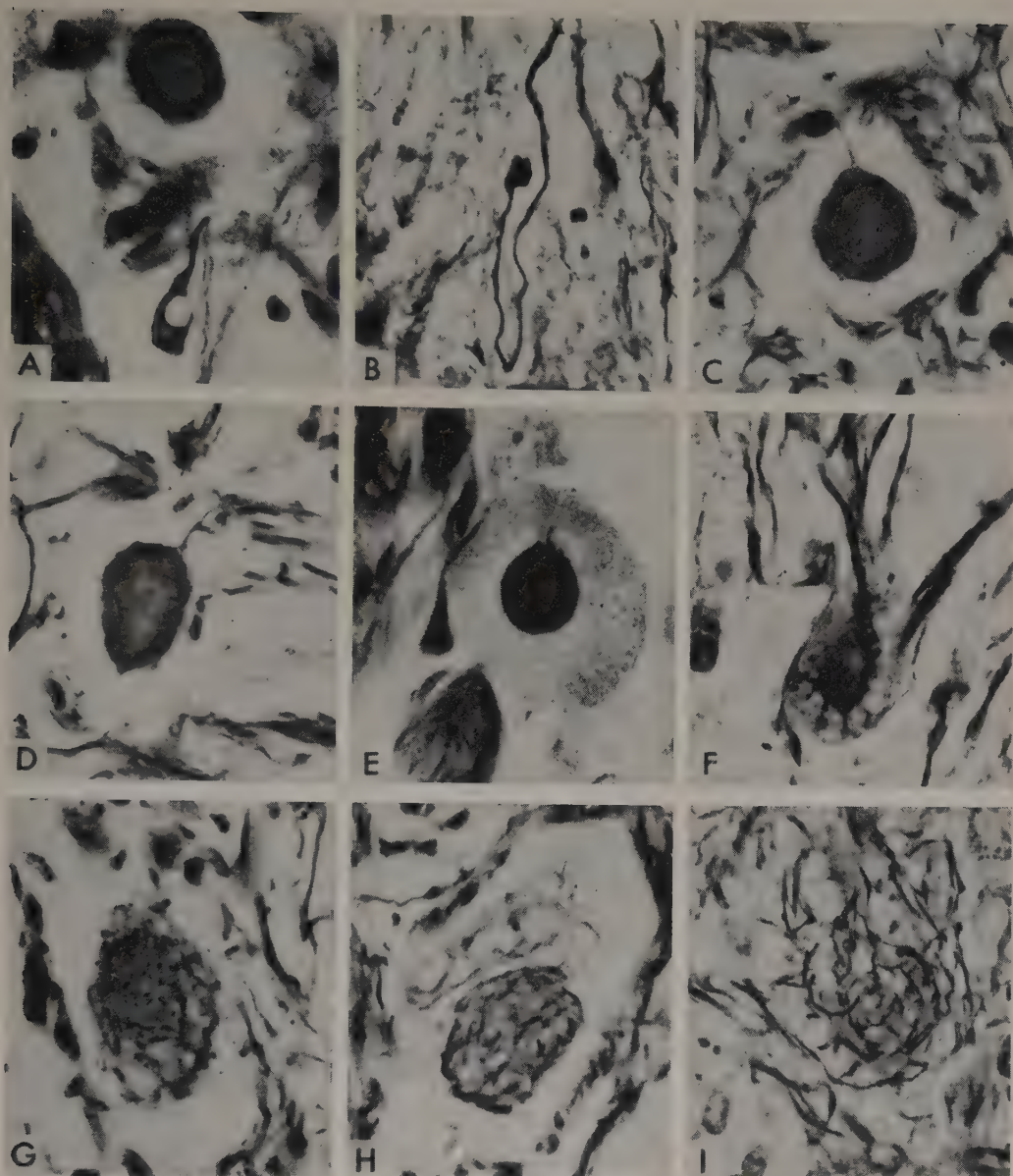


Fig. 132. Varicose thickenings (A—B) and nerve endings of different kinds (C—I) in the avian pituitary. Fig. F is from *Diomedea melanophris*, all other figures are from the same domestic goose (*Anser anser*).

A: varicose thickening in the neural lobe (below). B: Varicose thickening of a loop in the glandular layer of the median eminence. C—F: bulb-shaped endings in the neural lobe. In E and F the colloid mantle is distinct due to counterstaining in azan. The colloid mantle attaches to the bulb also when the latter is partly isolated in the tissue (E). G—I: more net-like structures in the neural lobe, in G and H surrounded by a colloid sphere.

All figures from 8 μ paraffin sections, impregnated according to Bodian, in A, B, E and F counterstained in azan. Fixation: Bouin. Magnification: A—H 820 $\times$, I 630 $\times$.

to the nerve ending and does not seem to be a simple fibre-free space in the tissue (fig. 132: E). The size of these nerve endings varies from a few μ to that of a cell, in exceptional cases even more. Also the shape is variable. The rounded contour is most common, though elongated and completely irregular endings may also occur in many glands. The compact appearance of the ending is typical of some glands, but in others there is instead a sphaerical net of delicate nerve fibres in the centre of some colloid masses, or a diffuse tangle of fibres without distinct contours (fig. 132). Another structure of a similar kind is characterized by a blackish body with spine-like, somewhat indistinct processes in the centre of a light colloid mass and continuous with a fibre. The latter type of nerve endings is often, especially in one preparation of *Diomedea*, of an elongated shape (fig. 132 F). Azan-blue colloid droplets surrounded by cage-like nets of nerve fibres are also common in some glands, but it is often difficult to see if the net is a true nerve ending or if two or more fibres are connected with it. The size of all these structures may vary from one third of a nucleus diameter to a few cell diameters. Structures interpreted as degenerating endings of the said kinds have also often been observed. They appear as bluestaining colloid masses, which in Bodian preparations contain a more or less distinct, black body without connection with nerves, or the centre of the colloid contains only scattered fragments of a silver-reducing matter.

The functional significance of these nerve endings in mammals has been interpreted in different ways. Vasquez-Lopez (1942), who found them in special processes from the neural lobe of the horse, thought they are sensory structures registering osmotic conditions in the blood. Hanström (1946, 1947) who found typical endings of this kind in the tiger and some other mammals suggested that they are related in some way to the transport of colloidal matter from the neurosecretory hypothalamic centres along the nerve fibres to the neural lobe, and a similar interpretation is given by Bargmann (1949). Finally, Stutinsky (1949) examined identical bulb-shaped nerve endings in the dog and concluded that they arise by invasion of nerve fibres into certain "cellules parenchymateuses" in the median eminence and the neural lobe. The intruding nerve fibre is said to form a net around the degenerating cell nucleus and finally to replace it, thus giving rise to a "bulb-shaped nerve ending" with surrounding remnants of the cytoplasm which is thus the colloid mantle.

When discussing the conditions in birds a number of facts must be considered. First, the number of nerve endings of this type is even in *Anser* exceedingly small compared with the number of nerve fibres entering the neurohypophysis. Further, many birds lack these nerve endings completely. This is, for instance, the case in more than ten pigeons in my collection, whereas I found a few poorly developed endings in two specimens. The endings are also extremely variable and often degenerating. This does not speak in favour of a sensory function, and this hypothesis is contradicted also by their situation as far from the blood vessels as possible. The typical bulb-shaped endings usually occur in the nerve fibre bundles and near the ependyma and never in the glandular zones.

It has not been possible to find any intermediate stages in birds indicating the development of the nerve endings from "cellules parenchymateuses" by invasion of nerve fibres. The argyrophilic bulb or net in the centre of the colloid mass never shows the structure or the staining properties of a nucleus when seen in azan or haematoxylin preparations. Further, there are hardly any cells from which such a development could start in birds, for no cells similar to the "cellules parenchymateuses" or "pseudoganglionnaires" in the dog have been observed. The exceptional nerve cells in two geese (p. 207) may be considered in this connection, but they are quite normal and have not been seen to degenerate. On the contrary, many small bulb-shaped nerve endings are much smaller than a cell nucleus and the surrounding colloid much smaller than a cell body. This does not speak in favour of the view that there had originally been an enlarged, degenerating cell.

Since a continuous series of stages from small endings or varicose thickenings with or without colloid mantles to typically developed and large nerve endings can easily be found, I must interpret the bulbs and nets as the result of an exceptional behaviour of the nerve fibres without partaking of other cells. The surrounding colloid may be liberated from the nerve fiber itself. The numerous varicose swellings and other abnormalities of the nerve fibres in the neurohypophysis make such a development plausible, and the fact that a colloidal matter is transported to the gland by the nerve fibres (cf. p. 255 ff.) makes the colloid mantle around the endings easy to explain. Bargmann has also shown in beautiful pictures how the Gomori-positive colloid can be aggregated around the nerve fibres in this way.

Although I thus agree in the same interpretation of the large nerve endings as Hanström and Bargmann, I wish to point out that they are certainly accidental structures of little importance to the function of the gland. This is shown 1) by their inconstant appearance and their tendency to degenerate, 2) by their relatively low number in the specimens in which they are present and 3) by their absence in many specimens. According to Cajal (1928), very similar large bulbs are formed by the ends of the fibres when a nerve is ligated for a longer time and around wounds in the central nervous system. The bulbs in the neurohypophysis may arise in an analogous way from fibres which have been checked in their development and they need not necessarily have a specific function.

Undoubtedly the functionally important innervation of the neurohypophysis in birds has the shape of a reticulum, sometimes with more specialized loops in the eminentia mediana. The peripheral innervation apparatus in the neural lobe is, thus, of the same type as the "ground plexus" which is the typical terminal structure of autonomic nerves in other parts of the body (cf. Hillarp 1946). If the fibres in the neural lobe normally terminate in free endings, these must be very simple and little conspicuous and have, anyhow, not been observed.

The Nerve Fibres of the Adenohypophysis and the Plexus caroticus

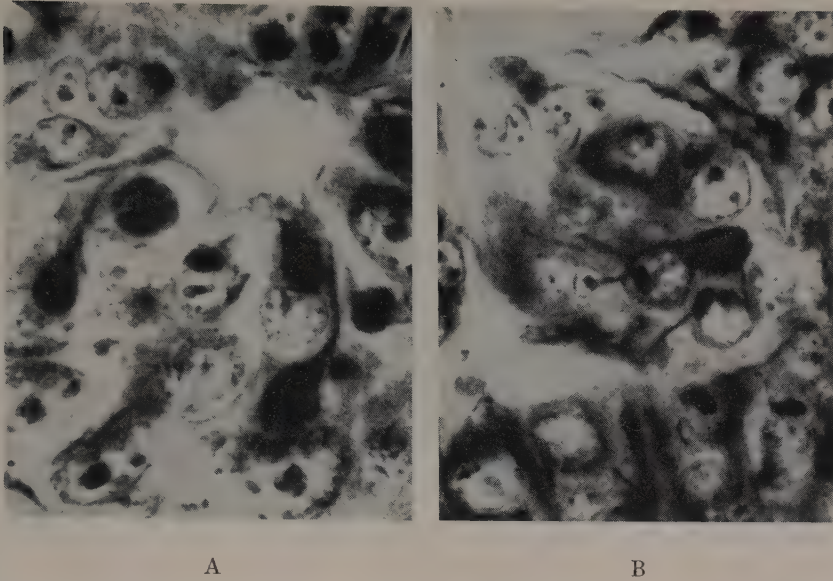
The innervation of the adenohypophysis is of essential importance to all discussions about the control of the function of the gland and will therefore be treated in detail.

Drager (1945) did not find any nerve fibres in the pars distalis but states that the pars tuberalis contains nerve fibres coming both from the tractus hypophyseus and from the vascular autonomic plexus. He admits, however, that the hypothalamic nerve fibres in the pars tuberalis are few and cannot be followed far into the glandular substance. He also followed the nerve bundles which run along the arteries of the pituitary region but stated that these bundles are independent of the pars distalis. In general, my own observations confirm Drager's statements except for a few details concerning the innervation of the tuberalis.

Green (1951) did not observe any nerve fibres in the adenohypophysis of the birds which he examined.

Since my investigations on the avian pituitary started, a considerable part of the time has been devoted to search for adenohypophyseal nerve fibres. Most of the silver impregnation methods mentioned in this and the preceding chapter were used mainly for this purpose. The Bodian technique, which is generally admitted to be one of the most reliable for investigations of this kind, was varied in different ways. The result was, however, practically negative, probably partly because I always compared critically with a Gömöry slide showing the non-nervous reticular fibres when the structures in the silver preparations were to be interpreted.

First a few words should be said about the result of some impregnation methods. If the pH of the silver proteinate used for Bodian impregnation is varied the result of the impregnation is highly altered. Thus, if the pH is lowered by addition of acetic or picric acid, the picture becomes granular and at a sufficiently low pH the delicate nerve fibres are not impregnated at all. If, on the contrary, the pH is raised by addition of ammonia or KOH the impregnation remains good as regards the particle size of the precipitated silver, but the result is less selective. Also glia and reticular fibres and finally also collageneous fibres become visible, whereas the true nerve fibres disappear. Thus, in some preparations impregnated at a high pH there is a rich system of delicate fibres around the glandular strings of the adenohypophysis, but a comparison with the reticular fibres in Gömöry preparations shows that the same structures have been impregnated in both cases. The reticular fibres are also often impregnated in Golgi, Cajal and Bielschowsky preparations and may be mistaken for nerve fibres, especially if the impregnation is less complete. In perfectly impregnated slides in which the reticular fibres are unstained the adenohypophysis contains no or very few fibres. The result is supported by the fact that indisputable autonomic nerve fibres *are* distinctly and completely impregnated in some exceptional cases when parts of the perivascular plexus has entered the pars distalis (cf. below). In this connection it should be mentioned that Green (1951 a) examined the fibres in the pars distalis of rabbit



A

B

Fig. 133. *Branta leucopsis*, structures similar to nerve endings in the pars distalis (central parts). In A pathologically enlarged cells with very large dark bodies in the plasma, in B two normal-sized cells with smaller dark bulbs, which are more like true nerve endings. Cf. text!

— Bouin, paraffin 10 μ , Bodian impregnation followed by azan staining. 800 $\times$.

and man by phase microscopy and found that they have a refractive index which is different from that of true nerve fibres. His result proves, with a high degree of certainty, that nerve fibres are absent or rare in the investigated glands.

One goose (*Branta leucopsis*, ♂ ad) reacted in such a way to Bodian impregnation that it must be mentioned separately. The adenohypophysis contains fibres which surround all the glandular strings. The glandular cells, especially those with a basophilic reaction in the plasma, contain one large black body each, and this body is continuous with a more or less regular black stalk which is directed towards the surface of the cell and can be followed in a few cases even outside the cell membrane (fig. 133). The continuity between these intracellular bulbs and the perilobular fibre system could not be stated, however. The picture strongly suggests a rich system of nerve fibres which enter the glandular cells and end in terminal bulbs closely attached to the nucleus. However, the specimen was not normal. Some glandular cells in the pars distalis are enlarged and have reached a size three or four times that of common cells. The "end-bulbs" in these abnormal cells are enlarged on the same scale (fig. 133 A). The fibre system around the glandular strings does not differ from the reticular fibres in Gömöry preparations of geese apart from large varicose swellings which are common. The impregnated fibres are continuous with a similar system in the pars tuberalis, but this is not connected with the fibres in the neurohypophysis nor with autonomic nerves from

the carotis. Certainly we have to do with the common non-nervous reticulum which has acquired peculiar chemical properties under the influence of the disease. If the peculiar intracellular "nerve endings" are carefully examined it is seen that they usually have ill-defined contours, and this is especially true of the stalks of the black bodies which form the connection with the outside. This is distinctly different from true nerve endings which are well defined structures with distinct contours. Maybe the structures suggesting nerve endings are disintegrating Golgi bodies which are discharged into the vessels, or perhaps they are a more direct effect of a parasitic infection. In Bodian slides of normal geese the Golgi bodies often appear as dark dots in the basophils but are never connected with external fibres, although a string from the dark body towards the surface of the cell can be seen in some cases.

Unmistakable nerve fibres are, however, present in the adenohipophysis of some birds, and they will be described here since they make the negative statement of a specific glandular innervation more significant. The nerve fibres in the adenohipophysis are always very few and are searched for in vain in many specimens. Since no nerve fibres leave the neurohipophysis, the fibres in the adenohipophysis must have their origin in the autonomic bundles belonging to the plexus caroticus, and in a number of cases this could be actually stated.

The autonomic nerve along the carotis can be followed in the canalis caroticus from the occipital region to the point where the arteria speno-maxillaris is emitted. Just behind this point it is intimately connected with the ramus palatinus of the n. facialis for a short distance, before this nerve leaves the bone together with the a. palatina (syn. Vidiana). The main portion of the carotic nerve leaves the bone together with the a. speno-maxillaris, and only one small bundle on each side follows the carotis into the sella turcica. Near the intercarotic anastomosis the two autonomic nerves split up in more fascicles on the surface of the carotis and pass two or more small sympathetic ganglia. Some of the latter are regularly situated on the surface of the anastomosis and may also be present between the surface of the vessel and the pituitary, so the ganglia may be expected to be concerned with the gland. I have followed these autonomic bundles in a number of pigeons, geese and one duck and in addition in a number of chick embryos. They become distributed in the walls of the venous caverns of the sella and part of them are always destined for the further course of the arteries. Also the capsule of the pars distalis is covered by a sparse system of such fibres.

A number of such fibres, in most pigeons only a few (less than 10) but in one goose a real bundle, enters the neural lobe together with the arteria hypophyseae inferior. However, this innervation is certainly of restricted value to the function of this part since the fibres are so few. They should certainly be regarded as the normal vascular innervation of the artery.

The pars tuberalis also contains scattered fibres of this kind in the geese and pigeons, but the number of fibres is small — usually less than 10 can be discovered in a section. This is not in perfect agreement with Drager's (1945) account, neither

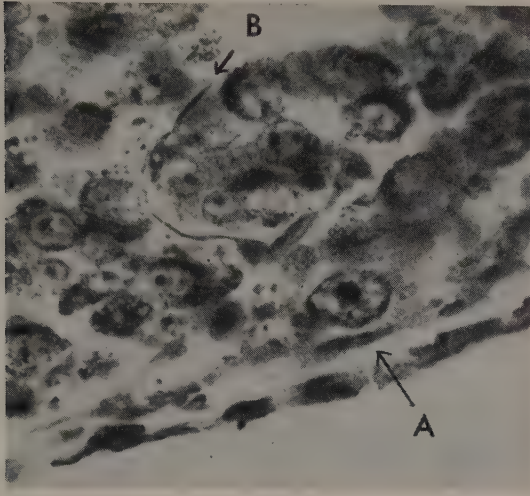


Fig. 134. *Anser anser*. Superficial part of the pars distalis. The large cell inside the capsule (A) is a sympathetic ganglion cell, and a nerve fibre belonging to the carotic plexus can be seen among the glandular cells (B). — Bouin, paraffin 8μ , Bodian impregnation. $760\times$.

can I confirm his statement that also hypothalamic fibres enter the pars tuberalis. There are no fibres which leave the eminentia in my preparations. Many of the superficial eminentia loops mentioned in the previous parts of this chapter may look like fibres leaving the eminentia and destined for the pars tuberalis, but a careful examination always shows that they turn back when they reach the intima piae. I therefore believe that these fibres have been misinterpreted by Drager.

The pars distalis also contains a few scattered fibres in most preparations of pigeons, and also in the geese and the ducks. The fibres are, however, so rare that large areas in a section must be examined before a single fibre is found. They are present both in good Bodian preparations and in Cajal preparations (here only such preparations are considered, in which the reticular system is entirely unstained). It has been seen several times that fibres in the pars distalis are continuous with the autonomic fibre bundles in the capsule of the organ, and the nervous character of the fibres can therefore hardly be doubted. The fibres in the pars distalis are, however, so few that they cannot be able to influence the function of the gland.

In the superficial parts of the pars distalis of two geese (*Anser anser*) there is a very rich net of nerve fibres restricted to smaller areas in the glandular tissue and even nerve cells are present there (fig. 134). Careful examination shows, however, that a part of the perivascular plexus of a venous cavern is dislocated into the glandular tissue at these places. The fibres are all continuous with the perivascular fibres of the venous wall. The cases are of interest since they show that autonomic fibres can be impregnated also within the glandular tissue of the pars

distalis. The largely negative result of the search for a specific glandular innervation must therefore be regarded as significant. The conclusion will be that if a specific glandular innervation exists in the adenohypophysis of birds, it must consist of fibres of a hitherto unknown kind which do not show the common properties of nerve fibres. And even though this possibility must be taken into consideration it should be borne in mind that the hypothetical nervous system in question has never been demonstrated.

CHAPTER XI

The Neurohypophysis and its Innervation in Birds Compared with that of Other Vertebrates

The comparative anatomy of the adult neurohypophysis was recently reviewed by Green (1951) in an interesting paper dealing also with the innervation and the vascularization of the pituitary. It is unnecessary therefore to survey these structures in non-avian vertebrate classes here, and I confine my discussion to some special problems.

Comparison with Reptiles and Mammals

The structural differentiation of the neurohypophysis in mammals, birds, and reptiles agrees so closely that comparisons can be made with a high degree of certainty. The neural lobe, which is formed by proliferation of the top of the saccus infundibuli, is undoubtedly homologous throughout. The term infundibular stem is regarded by Green (1951) as little defined and he suggests that it should be used for descriptive purposes only and not for comparative work. However, if the stem is formed by the basal part of the saccus infundibuli in all amniote embryos, it can be regarded as homologous. Since the stem is often indistinctly delimited from the eminentia mediana, especially in lower forms, it is sometimes necessary to follow Green's suggestion and treat the stem as a part of the eminentia mediana when comparisons are made between adult animals.

The eminentia mediana can hardly be homologized directly in different amniotes. This part has been defined by Green (1947) as that part of the neurohypophysis which is coextensive with the primary capillary net of the hypophysioportal system, and he also remarks that this part has a typical histological appearance. In his paper of 1951 Green gave similar definitions and pointed out, that the eminentia mediana has an exact topographical meaning in different animals if it is defined in this way. This is, of course, valuable, but it should be pointed out that Green's concept is a functional unit rather than a comparative morphological one, and it will be discussed below whether it is homologous throughout or not.

Nowakowski (1951) defines the "infundibulum" in the cat as that part of the diencephalic floor which is coextensive with the pars tuberalis. He also remarks,

however, that the "infundibulum" is distinctly delimited from the surroundings by a sulcus infundibularis, and that it is characterized by its histological structure which is different from that of the adjacent parts of the brain. Since Nowakowski also observed the dense capillary net on the surface, his "infundibulum" must correspond perfectly to the eminentia mediana as defined by Green and as it has been described in the present paper.

The chief characteristic used by Nowakowski for defining the eminentia ("infundibulum"), viz. the extension of the pars tuberalis¹ can not be used for comparative purposes, although it happens to coincide with the eminentia mediana in the cat and perhaps in some other mammals, for it is not even coextensive with the eminentia in all mammals (fig. 137). In birds the pars tuberalis extends far up along the sides of the tuber cinereum and is often absent from the central and most typical parts of the eminentia mediana. In snakes there is no tuberalis at all but still a distinct and typical eminentia, characterized by the same histological structure and the same vascularization as in birds and mammals (author's material). Nor is there any distinct sulcus infundibularis in many reptiles and birds, so this characteristic is also useless for comparative purposes.

Nowakowski (1951) also found a very characteristic histological differentiation in the infundibulum of the cat, and his description agrees closely with the structure of the avian eminentia. In the cat the eminentia differs from other walls of the brain in the absence of the common felty and unordered superficial glia zone and in the presence of a "periphäre Zone" which has a structure perfectly like the glandular layer in birds. In a great number of reptiles and birds I have been able to ascertain that the extension of this characteristic glandular layer coincides with the extension of the primary plexus of the hypophysiportal system of blood vessels, and this seems to be the case also in amphibians. Although Green's definition of the eminentia is adequate it can thus be made easier to use in practice by adding some histological notes (cf. also Weaver and Bucy 1940). It would then be defined as that part of the diencephalic floor which is coextensive with the primary plexus of the hypophysiportal system of blood vessels, and which is also characterized by a superficial layer (glandular layer) which differs histologically from adjacent superficial parts of the brain. The histological characteristics of this glandular layer are the arrangement of the glia fibres perpendicularly to the surface, and the total or partial absence of cell nuclei and coarse nerve fibres. This histological appearance of the eminentia is somewhat

¹ Nowakowski (1951) repeatedly emphasizes the inadequacy of the term *pars tuberalis*, which is independent of the tuber in the cat and is coextensive with Nowakowski's "infundibulum" (= eminentia mediana). He therefore uses the term "*pars infundibularis*" for the epithelial cover of the infundibulum. In birds, however, the *pars tuberalis* extends for a considerable distance along the sides of the tuber cinereum (fig. 33, 34), and thus the term is not inadequate in this group. I prefer the term "*pars tuberalis*" which has been used only in one sense by recent investigators, in contrast to the term "*pars infundibularis*" which has been used extensively also for the part generally called *pars intermedia*.

obscured by complications of the surface in some mammals, especially in man, but it is always distinct from the structure of the surrounding tuber cinereum.

The eminentia mediana (Green) and the infundibulum (Nowakowski) are thus undoubtedly synonymous expressions for the same structure. The vascular system and the histological appearance which are used as criteria in the definitions are so intimately connected with the function, however, that the structure defined must be regarded as a functional unit rather than a comparative morphological one. This will be clear when the extension of the eminentia in different amniotes is compared.

The ventral wall of the third ventricle from the chiasma (supra-optic decussations) rostrally to the saccus infundibuli caudally is well defined and necessarily homologous in all amniotes. In the adult cat there is an eminentia which covers most of this surface with the exception of a small part just behind the chiasma, which does not show a neurohypophyseal structure. This area is called *pars oralis tuberis* by Nowakowski (1951), and the same author points out that the *pars oralis tuberis* is still larger in the rabbit (l. c. p. 264). In birds the whole ventral wall from the chiasma to the saccus is differentiated as an eminentia, except in the duck and the goose in which a small *pars oralis tuberis* is left just behind the chiasma. In reptiles, especially in lizards and snakes, the eminentia occupies a very small part of the post-optic diencephalic floor, and the *pars oralis tuberis* thus extends far caudally from the chiasma. If we turn to the amphibians the *pars oralis tuberis* is very large, and the eminentia is restricted to a minute portion near the pituitary (cf. Green 1947 a, fig. 1). The eminentia or "infundibulum" can therefore hardly be homologous throughout, but we must accept the fact that very different parts of the post-optic diencephalic floor can be differentiated as an eminentia in different animals.

The histological structure of the neurohypophysis was said to vary extensively in birds, and the same holds good for amniotes in general.

The variations of the neurohypophysis in reptiles are even greater than in birds. The simplest structure of the neurohypophysis is found in *Sphenodon* (Wyeth and Row 1923), in some lacertilians (e. g. *Xanthusia*, *Gerrhonotus*, *Coleonyx*, *Tarentola*, *Hemidactylus*, *Gambelia*, *Uta*, *Uma*, *Streptosaurus*, *Dipsosaurus*, *Lacerta*, *Cnemidophorus*, *Mabuia*, *Eumeces*, *Chamaeleon*, author's coll.), and in some chelonians (author's coll.). The neural lobe of these reptiles is a simple, more or less thin-walled, in many species bilobed, or sometimes wrinkled sac. Its wall consists of an ependyma with processes towards the external surface, of a fibre layer and of a glandular layer. Pituitocytes are entirely absent in most species (fig. 135, 140). In a few of the said lizards which have slightly thickened walls in the neural lobe there are some cells which have left the ependymal layer, but their number is negligible. The eminentia mediana is slightly thickened but has practically the same structure. In a few species some ependymal cells have left the ependymal layer also in the eminentia and are situated near the ventricle as in *Columba* (fig. 110 B). An infundibular stem is often recognizable morphologically, but its structure

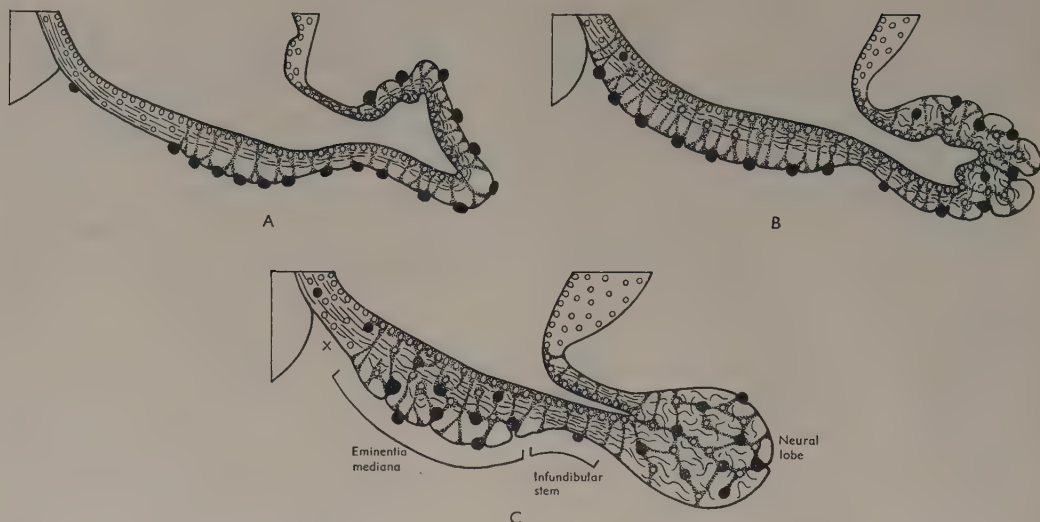


Fig. 135. Diagrams showing the histological differentiation of the neurohypophysis in amniotes. In A a primitive structure (most Lacertilia, *Sphenodon*), in B the structure in the majority of birds, in C the mammalian type. The processes of ependymal cells, pituicytes and glia cells are stippled, the blood vessels are black, and the coarse nerve fibres are indicated by lines.

Connective tissue elements are not considered. X = pars oralis tuberis.

is the same as that of the neural lobe and the eminentia, though the wall is often a little thinner.

This "*Sphenodon*" type of neurohypophysis is very simple. There is practically no histological difference between the eminentia and the neural lobe, but all walls have a uniform structure. In my opinion this type may be regarded as primitive also from a phylogenetic point of view, partly because it can serve as a basis for the development of more complicated types, and partly because it reminds of the embryonic neurohypophysis in amniotes. Its phylogenetic primitiveness is supported also by the fact that it is present in *Sphenodon*, which has retained a lot of other primitive features, and by its tendency to be preserved in other lower amniotes.

In other reptiles the neural lobe is more complicated. In snakes (13 genera examined) the neural lobe is practically compact and the recessus infundibuli stops at the base of it with a pair of very small lateral pouches. Pituicytes are fairly frequent. In *Python* the lumen is a little larger, but the neural lobe is still very compact. The eminentia mediana is thick with invaded cells and in many species with a folded surface so that the portal capillaries are buried in the tissue. The stem is often histologically distinct, especially in *Python* in which the wall is entirely occupied by the large bundles of the tractus hypophyseus. Also some lizards have a similar complicated neurohypophysis, especially *Varanus* and *Zonurus* (author's coll.), but the lumen is larger in the neural lobe. May be the size of the organ partly explains why it is so complicated in these two species, but this does not seem to be the general rule in vertebrates. *Riparia*, which is a

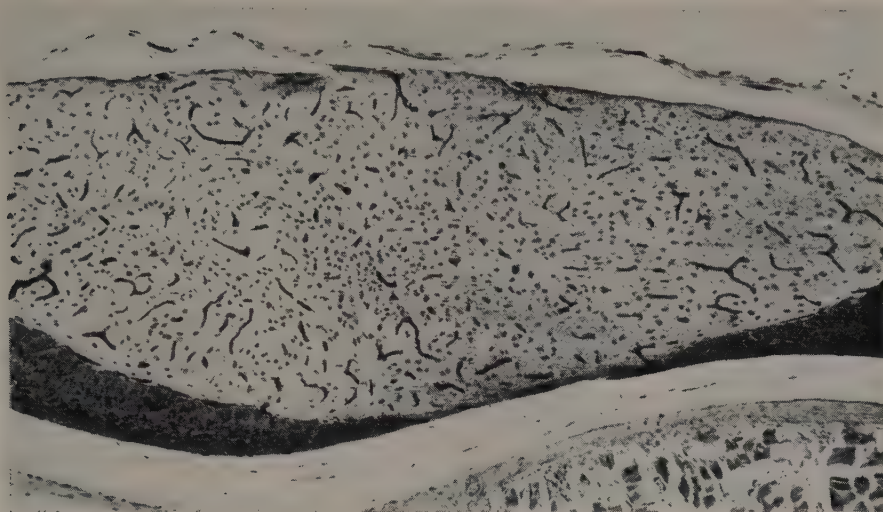


Fig. 136. *Nyctalus noctula*, cross section of the neural lobe with surrounding intermedia. Note that the lobe is compact, the pituicyte nuclei are scattered all over the lobe and the vessels which are black with ink are present everywhere. — Ink injection, Bouin, paraffin 10μ , haematoxylin of Ehrlich and eosin. $110\times$.

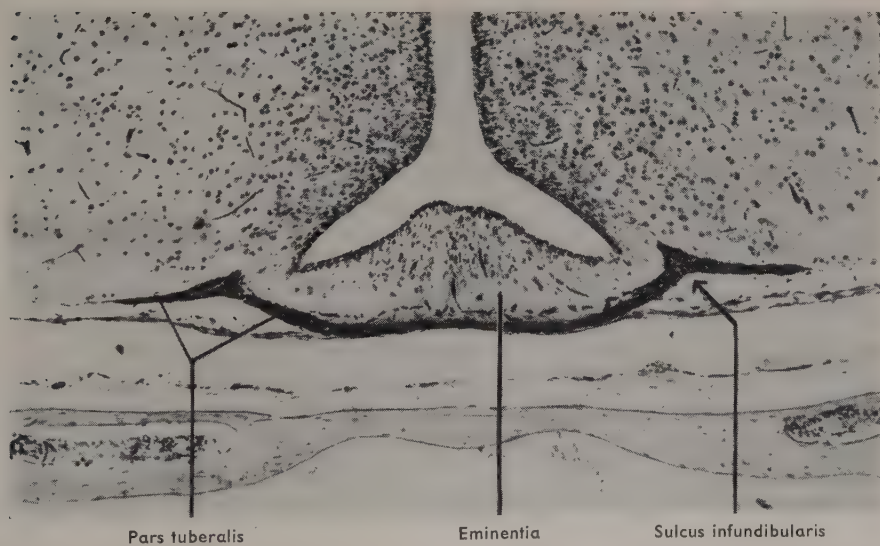


Fig. 137. *Nyctalus noctula*, transversal section through the eminentia mediana. The pars tuberalis does not stop at the sulcus infundibularis but extends to parts of the brain outside it. The ink-injected vessels are not restricted to the surface of the eminentia but pass through the glandular layer, and also form loops towards the lumen. — The same technique as fig. 136. $110\times$.

small bird, has a very complicated neural lobe, whereas *Diomedea* has a simple one, and *Sphenodon* has a primitive neural lobe in spite of a considerable size.

We are thus able to state that the neurohypophysis in reptiles show variations of similar kinds as those stated for birds. The primitive type of neurohypophysis found in *Phasianus*, *Gallus*, *Diomedea* and owls may be directly compared with the type found in *Sphenodon* and many lizards, whereas the advanced type found in *Anser*, *Larus* etc. is similar to that in snakes. The histological variation in reptiles is, however, still greater than in birds, and I hope I shall be able to show this in a future paper.

The mammalian neurohypophysis is invariably of a more complicated type. The most primitive structure is found in the Monotremata, described by Hanström and Wingstrand (1951). In *Tachyglossus* the eminentia mediana is very schematical with a thin wall and with the capillaries practically restricted to the external surface. All three layers described in birds are present and distinct: glandular layer, fibre layer and ependymal layer. The surface of the eminentia is smooth and uncomplicated, but some cells have left the ependyma and are situated as glia cells within the wall. There is no distinct infundibular stem, but the neural lobe has thick, proliferated walls in spite of the fact that the lumen is well preserved. In the other genus, *Ornithorhynchus*, the lumen of the neural lobe is highly reduced and the infundibular stem is more distinct, but the eminentia mediana is thin and schematical as in *Tachyglossus*.

The marsupial and placental mammals have a much more complicated neurohypophysis (fig. 136, 137). The neural lobe is compact with the exception of the families Felidae and Ursidae among the carnivores (Hanström 1947). There is a distinct infundibular stem characterized by a preponderance of the fibre bundles of the tractus hypophyseus in the wall, and this part, too, is often compact. The eminentia mediana is extremely complicated in man (Christ 1951, Green 1948), and it seems difficult or even impossible to trace the primitive pattern described above for reptiles and birds in the human material. When looking through professor Hanström's collection at this institute I ascertained that the eminentia of other mammals shows considerable complications if compared with reptilian and avian material, but the primitive sauropsidan pattern could be traced in the majority of species. The wall of the eminentia is thick, the surface convoluted, and its capillaries are often buried deep in the nervous tissue (cf. also Green 1951, p. 261, 274). The wall of the eminentia also contains numerous glia cells just as in the complicated types of eminentia in birds (fig. 137). The mammalian neurohypophysis is, thus, comparable with the advanced type found in snakes and geese, for instance, but it is sometimes still more advanced since the lumen is reduced also in the infundibular stem of some species (e. g. Ungulates, man).

A happy chance brought into my hands Nowakowski's (1951) excellent description of the pituitary of the cat, just as I was writing this chapter, and this opened a possibility of detailed comparison with a mammal. It is striking how much in Nowakowski's paper agrees with the conditions in birds as described in the

preceding chapters, but this may partly depend on the fact that the cat has a fairly primitive neurohypophysis with a large lumen in the neural lobe and with a fairly simple eminentia. Some of the points of agreement will be mentioned here, but the reader is referred to the original. In fact some parts of my descriptions in chapters IX and X appear to be direct translations of Nowakowski's text, but it should be pointed out that I had not seen Nowakowski's paper when these chapters were written. It should be remembered, however, that the terms are slightly different. Nowakowski's infundibulum is the same as my eminentia mediana, his radix infundibuli is my transitional zones, his pars infundibularis is my pars tuberalis, and his Zwischenstück is my infundibular stem. Nowakowski's theoretical deductions concerning the function of the neurohypophysis will be criticized in the following chapters.

Nowakowski found two layers in the eminentia mediana, one external ("periphere Zone") characterized by the absence of cell nuclei and coarse nerve fibres, and corresponding to my glandular layer. His "zentrale Zone" is the same as my fibre layer and ependymal layer.

Nowakowski found a specific innervation of the "periphere Zone", consisting of delicate fibres which are said to arrive from nearby parts of the tuber nuclei, the nuclei tuberis infundibularis. These nuclei must correspond anatomically to the ventral parts or more of the nuclei tuberis in birds. The statement in Nowakowski's paper is thus beautifully corroborated by the result of the present work, that the glandular layer in birds is innervated mainly from the nuclei tuberis.

Nowakowski also used the Gomori haematoxylin and showed that the "periphere Zone" in the cat eminentia has little or nothing to do with the neurosecretory neurons of the tr. supraoptico-hypophyseus, which mainly terminates in the "Hinterlappen" (neural lobe). This is exactly the same result as that obtained for birds in the present paper.

Nowakowski describes a "Radix infundibuli" which is a transitional zone between the "Infundibulum" and the tuber cinereum. It is characterized by a neurohypophyseal structure along the surface, but by the presence of the nucleus tuberis in the deeper layers. This corresponds exactly to the descriptions of the transitional zones of the eminentia in the present paper.

Many other identical features in the distribution of the nerve fibres and in the appearance of glia and connective tissue in the neurohypophysis could be pointed out between the cat and birds, but the said statements may suffice to show that there is a fundamental plan in the histological structure of the neurohypophysis, common to birds and mammals.

The innervation of the amniote pituitary has been carefully reviewed by Green (1951). The tr. supraoptico-hypophyseus is the tract most easily identified and has been followed to its origin in the supraoptic nucleus in representatives of all amniote classes. My Bodian and Gomori preparations of *Tropidonotus natrix* show this tract very clearly, and it could be stated that it is destined mainly for the neural lobe as in birds and mammals. As pointed out especially by Kuhlenbeck

(1937) and Meyer (1935) the nucleus supraopticus is undoubtedly homologous in all amniotes, and it is interesting to note that it has a neurosecretory function and is associated with the neurohypophysis in all the investigated species.

The other fibre tracts ending in the neurohypophysis are insufficiently known in reptiles. Green (1951) distinguishes anterior, posterior and lateral portions of the pituitary fibre system, and he believes that the hypothalamic connections are very similar to those in higher vertebrates.

In mammals as well as in birds the nearby part of the tuber nuclei is connected with the superficial parts of the eminentia (cf. above). Also connections with the magnocellular paraventricular nucleus, with the lateral tuber nuclei, with scattered small periventricular cells and with the mamillary region have been demonstrated more or less convincingly in mammals (cf. Laurelle 1934, Romeis 1940, p. 463 ff., Harris 1948). Some of these connections may correspond anatomically to the systems described above for birds, but reliable conclusions seem difficult at present.

The histogenesis of the reptilian neurohypophysis seems to be practically unknown. In my preparations of viper embryos (*Vipera berus*) it could be seen that the compact neural lobe in this species is formed from the point of the saccus infundibuli by a kind of proliferation similar to the "diffuse emigration of cells" in birds. However, the mixing of nervous and mesenchymatic components is not so intimate as in birds. In a few embryos of *Anguis* and *Lacerta* there are no radical processes in the saccus infundibuli. This is natural, since the change from the embryonic saccus to the adult neural lobe mainly requires a slight thickening of the walls and an invasion of nerve fibres in these species.

Numerous embryonic investigations show that the neural lobe in mammals is formed by proliferation of the saccus infundibuli (cf. literature in chapter V), but details are few. Shanklin's (1940, 1944, and 1944 a) studies of the histogenesis of the neurohypophysis show that the differentiation of the pituicytes takes place in a similar way both in *Homo*, *Sus* and *Gallus*. The descriptions of the histogenesis of the pig neurohypophysis leave the question open, whether a "diffuse emigration" of nervous matter takes place in this animal. Anyhow no such process is described.

Comparison with Lower Vertebrates

As pointed out by Green (1951) the neurohypophysis of the amphibians can be successfully compared with that of the amniotes. There is an eminentia mediana with a capillary net which is the primary plexus of a more or less differentiated hypophysio-portal system of vessels. There is also a small neural lobe with an independent blood supply. I have been able to corroborate these statements in my material of *Rana*, *Bufo* and *Salamandra*. The homology of the neural lobe with the equally named structure in higher vertebrates seems unquestionable (cf. also Green 1947 a). The eminentia was briefly discussed on p. 239.

The fishes and cyclostomes are far more difficult to compare, and I enter into a discussion of these matters mainly because some previous statements bearing upon

avian material must be commented on. Tilney (1915, 1938) made a general comparison of the neurohypophysis in all vertebrates and he concludes that the dorsal parts of the neural lobe in birds is homologous with the saccus vasculosus in fishes and cyclostomes. The presence of a saccus vasculosus in amniotes and amphibians has, however, been absolutely denied by most recent authors (e. g. Pokorny 1926, de Beer 1926, Green 1951). The disagreement between different authors seems, however, to depend on confusion as regards the concept of homology and, moreover, on the more or less functional aspect in their comparisons. If the saccus vasculosus is defined as a thin-walled sac from the posterior part of the hypothalamic region, characterized by the curious primary sense cells described by Dammermann (1910) and without secretory function, there is no saccus vasculosus in the pituitary of higher vertebrates. A similar definition has probably played a rôle for the authors who deny the presence of a saccus, for they always support their opinion by histological finds. Such a definition is, however, functional and has little to do with homologies.

If reliable homologies are to be established the embryonic history of the organ must be considered, and this was tried by Tilney (1915). Then it will be possible to state whether the neural lobe and the saccus vasculosus represent identical parts in the structural pattern of the vertebrate hypothalamus, i. e. whether they are homologous. Such a statement must be independent of functional specializations in the organs.

Tilney's (1915) opinion that the processus infundibuli, the saccus infundibuli of the present paper, is homologous in all vertebrates is very difficult to disprove. Tilney recognized a dorsal or saccular surface and a ventral or pituitary surface in the saccus infundibuli (fig. 138). The ventral or pituitary surface is said to give rise to the neurohypophysis in fishes and the saccular surface is said to form the saccus vasculosus. The hollow and wrinkled neural lobe in some reptiles, in *Gallus* and *Botaurus* is said to be formed mainly by the saccular surface and would thus be homologous with the saccus vasculosus. The pituitary surface is said to form a slightly thickened, ventrally situated wall in the same species. The avian neural lobe would, then, be composed of a larger part homologous with the saccus vasculosus of fishes and a smaller ventral part homologous with the "neurohypophysis" of fishes. In mammals Tilney could find very few remnants of a saccus vasculosus. The bladders and clusters of epithelial-like cells in the neural lobe of a dog (1938, fig. 17 and 18) are mentioned as possible criteria for the presence of a saccus vasculosus but are certainly invaded intermedia strings, a possibility also mentioned by Tilney.

First a few points in Tilney's papers should be commented upon since they will easily give rise to mistakes. Whereas the figures in the paper of 1915 are carefully made, the figure of a bird pituitary in the last paper (1938, fig. 15) has very little in common with a normal bird pituitary at any stage of development (cf. figures in chapter VIII of this book). Further, it is impossible to find a different development of the "pituitary" and "saccular" surfaces of the saccus infundibuli in the

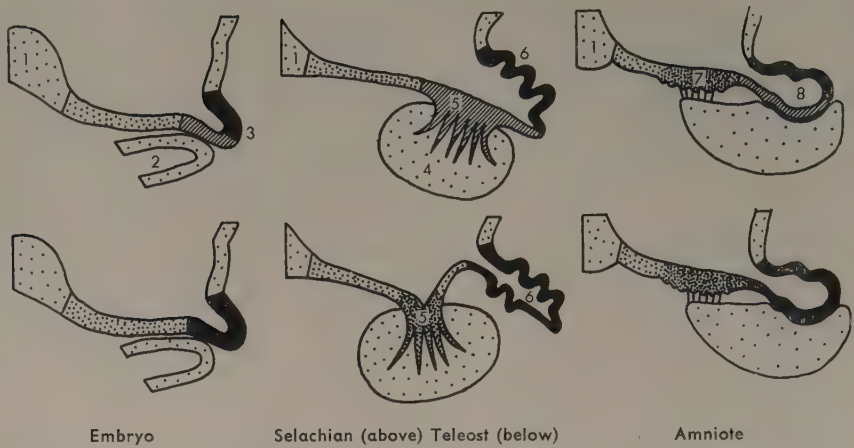


Fig. 138. Diagrams illustrating the probable homologies in the neurohypophysis according to Tilney (top series) and also the interpretation suggested especially for teleosts in this work (bottom series).

1 = chiasma opticum, 2 = Rathke's pouch, 3 = saccus infundibuli, 4 = adenohypophysis, 5 = "neurohypophysis" in the fishes, 6 = saccus vasculosus, 7 = eminentia mediana of the amniotes, 8 = neural lobe.

five bird species examined in the present investigation. The neural lobe arises from the top of the saccus infundibuli and from its lateral evaginations, either this occurs by formation of hollow buds and results in a sac-like wrinkled structure, or it takes place by diffuse emigration of nervous matter and results in a compact body. This also shows that the more or less sac-like appearance of the neural lobe cannot be used as a criterium for the presence or absence of a saccus vasculosus as Tilney evidently thought. This would, besides, result in the conclusion that *Gallus* and *Phasianus* have a saccus vasculosus, whereas *Perdix* has not. Such a conclusion is too improbable to be discussed.

Another possibility for homologization should be considered in this connection. As pointed out by several investigators of fish pituitaries (cf. Green 1951) the contact between the "neurohypophysis" and the adenohypophysis in fishes is very intimate. The "neurohypophysis" has long branches which enter the glandular tissue, and its fibres partly continue into the epithelial parts. Further, there is a common vascular system for the "neurohypophysis" and the adenohypophysis whereas the saccus vasculosus often has a separate blood supply (Green 1951). The most intimate contact between the epithelial and the nervous parts is, thus, between the "neurohypophysis" and the adenohypophysis, and this way is evidently the most important or the only one in which impulses from the brain can be transferred to the epithelial part. In amniotes and amphibians the most intimate and constant contact is between the eminentia and the adenohypophysis, mediated by the portal vessels and the pars tuberalis (cf. chapter XII and XIII). It is thus tempting to regard the neurohypophysis of fishes as the homologon of the eminentia mediana of higher verte-

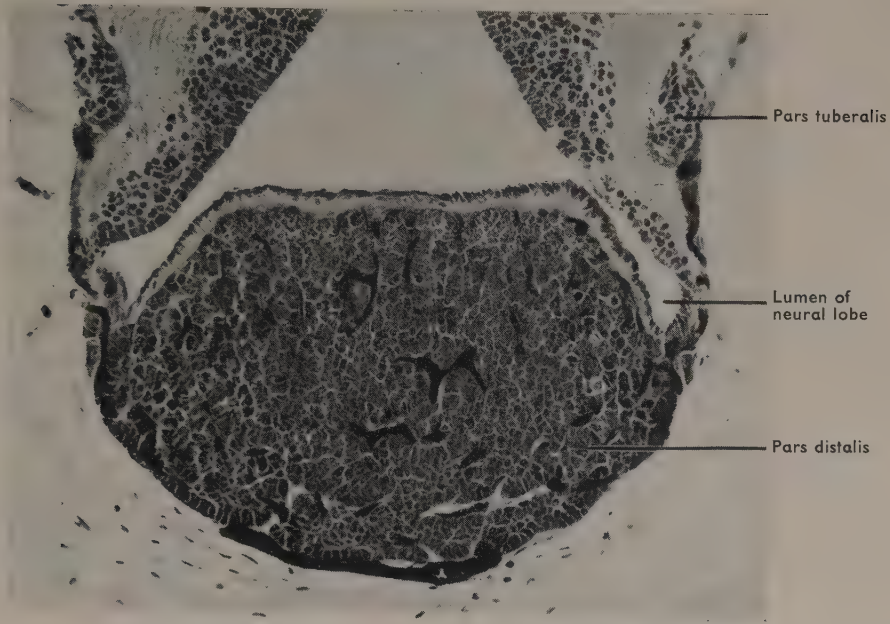


Fig. 139, *Salamandra maculosa*. Horizontal section of the hypophysis, showing the lateral diverticula of the ventricle, which are intimately associated with the lateral parts of the neural lobe. — Bouin, paraffin 10 μ , azan. 90 $\times$.

brates and the saccus vasculosus would then be completely homologous with the neural lobe. A similar interpretation of the neural lobe was suggested by Haller (1898). This hypothesis is not very stable, however, for it is supported by facts which have a distinctly functional character, and further the eminentia mediana was shown above to be a functional unit with a somewhat variable morphological significance (fig. 138).

The facts speaking for or against the different interpretations will be summarized below:

1. Haller's and Tilney's view that the saccus infundibuli ("processus infundibuli") is homologous in all vertebrate embryos is supported by its constant origin in the postero-ventral wall of the hypothalamic area, below the recessus mamillaris and below a more or less distinct evagination which Tilney calls post-infundibular eminence. A very strong support to the homology is the further differentiation of this pouch. It was shown for birds in chapter VIII that the saccus infundibuli gives rise to a pair of lateral pouches (primary branches) immediately after its appearance in the embryos, so that it becomes bifid at its end. This feature is often indistinct in the adult. In many lizards this paired appearance of the saccus infundibuli is preserved also in the adult (fig. 104), and the same is true for *Sphenodon* (Wyeth and Row 1923). In snakes the recessus infundibuli extends into a pair of lateral diverticula

which are especially distinct in *Python* (own observ.), and the neural lobe of *Tachyglossus* has also a paired appearance in the adult (Hanström and Wingstrand 1951). My preparations of adult *Salamandra maculosa* show that the laterally pointing tips of the neural lobe are hollow (cf. fig. 139), but they are compact in adult *Rana* and *Bufo*. Tilney (1915) observed these lateral pouches in embryos of birds and sharks. A rapid review of the literature shows that such a bifurcation of the saccus infundibuli takes place very early and is very distinct in the sharks *Scyllium canicula*, *Spinax niger*, and *Scymnus lichia* (cf. figures in Alexander 1927 and Wenig 1928), and it was observed also in *Raja* (Dammerman 1910, p. 674). The two lateral pouches are so distinct and large in *Scyllium* that it was discussed whether they were the result of an abnormal duplication or not. An examination of several specimens showed that there were large lateral branches also in normal cases. A paired development of the saccus infundibuli is typical also in *Petromyzon* (cf. below) and could be traced in *Muraenoids* (Boeke 1913).

It was suggested by Dammerman (1910, p. 681 and 687) that the process which gives rise to the neural lobe in higher vertebrates ("recessus hypophyseos") is different from the process which forms the saccus vasculosus in fish ("recessus saccularis"). The detailed agreement in the morphogenesis described above may be sufficient to show that they are homologous. Dammerman's opinion seems to be based mainly on the fact that the "recessus hypophyseos" lacks the specific sense cells typical of the saccus vasculosus, and this criterium cannot be decisive for a morphological comparison.

Haller v. Hallerstein (1934, p. 127—128) is of the same opinion as Dammerman, and he regards the wrinkled appearance of the walls in the saccus as a criterium against the homology with the neural lobe. This criterium does not hold, however, for many reptiles and birds have a neural lobe of this appearance (fig. 116). Further Haller v. Hallerstein means that a saccus vasculosus and a neurohypophysis exist simultaneously in some teleosts. It is true that the hollow processes of the "neurohypophysis" in some teleosts may look like the lobus nervosus in higher vertebrates, but this interpretation is not supported by embryological investigations. In selachians, which are sufficiently known embryologically, the development speaks in favour of a homology between the saccus vasculosus and the neural lobe of higher vertebrates, and there is little reason to believe that it should be different in other fishes. The teleosts may in this as in many other respects be deviating and difficult to interpret.

2. According to Tilney (1937, 1938) the "processus infundibuli" in *Petromyzon* is distinctly bifid at the posterior end, and this is also proved in the figures (cf. fig. 105 in this book). Moreover, the two branches are surrounded by a sheath of the adenohypophysis in a way which is indistinguishable from the conditions in many lizards with a primitive neural lobe invested by an intermedia. Tilney also maintains that he is able to homologize the different parts of the adenohypophysis, including the intermedia, from their ontogenetic development in *Petromyzon*. It is difficult to escape the impression that the "processus infundibuli" in *Petromyzon*

and its epithelial cover are homologous with the neural lobe and the intermedia in higher vertebrates (fig. 104, 105).

3. The innervation of an organ is usually regarded as an important criterium for homologies. One system, viz. the tractus supraoptico-hypophyseus resp. the tr. praeoptico-hypophyseus in Anamnia is undoubtedly homologous and is thus of interest to the homologies in the neurohypophysis. Palay (1945) has described this tract in the teleosts *Ameiurus nebulosus*, *A. melas* and *Noturus flavus* and stated that it is a neurosecretory pathway just as is the tr. supraoptico-hypophyseus of amniotes. Bargmann and Hild (1949) have studied the secretory activity in the tr. praeoptico-hypophyseus of the tench (*Tinca tinca*) using the Gomori haematoxylin. Because of the origin of the tr. praeoptico-hypophyseus in the praeoptic nucleus, which undoubtedly contains the homologon of the n. supraopticus of higher vertebrates (Meyer 1935), and because of its course dorsally of the chiasma to the hypophyseal region this tract is certainly homologous with the tr. supraoptico-hypophyseus in higher vertebrates. This conclusion is naturally supported by its neurosecretory character in many forms. Now several investigators have shown that this tract ends in the "neurohypophysis" of fishes (Green 1951, Palay 1945) and its secretions are evidently deposited there (Bargmann and Hild 1949). In amniotes this tract ends in the neural lobe. This speaks in favour of a homology between the neurohypophysis in fishes and the neural lobe in amniotes.

The fact that the saccus vasculosus and the "neurohypophysis" in fish often have a separate blood supply just as the neural lobe and the eminentia in mammals can hardly be of any great morphological significance, since the vascular system in general is rather variable. It will be shown that the origin of the neurohypophyseal vessels in birds may be highly different, and still greater variations must be expected when amniotes and fish are compared.

The points 1—3 above allow some considerations although definite conclusions can hardly be made. In my opinion the early morphogenesis of the neurohypophysis (point 1) must be of primary importance to homologies, the more so as it is characterized by a striking constance throughout. Point 1 thus seems to justify the opinion that the saccus infundibuli is homologous in all vertebrates. This means that the morphological pattern of the neurohypophysis is fixed and can be compared from one group to another.

In amniotes and amphibians the saccus infundibuli becomes the end-station of the tr. supraoptico-hypophyseus and at the same time becomes the site of some neurohypophyseal functions, whereas the ventricular floor between the chiasma and the saccus is partly or entirely differentiated as an eminentia mediana with another set of functions.

In fishes the conditions are not equally clear, and detailed investigations of the morpho- and histogenesis of the neurohypophysis would undoubtedly favour the discussion, since precise literature records seem to be scanty. The saccus vasculosus, if present, is formed by the saccus infundibuli (cf. Dammerman 1910, Smith 1914, Tilney 1915, 1938, Alexander 1927, Wenig 1928) and its homologon

in higher vertebrates is thus the neural lobe or parts of it. The saccus vasculosus has a distinct histological structure which cannot be refound in amphibians and amniotes. Its walls contain peculiar sense cells indicating that it is a sense organ (Dammerman 1910). This functional differentiation does not, however, change its homology with the neural lobe.

Whether the "neurohypophysis" in fishes is formed constantly by the "pituitary surface" of the saccus infundibuli as said by Tilney seems somewhat uncertain. The structure of the adult gland suggests that this is the case in some elasmobranchs and in *Protopterus* (Tilney 1915, Dawson 1940). In some teleosts such as *Lophius* (Green 1951), *Amia*, *Lepidosteus* and *Acipenser* (Kerr 1949) the situation of the "neurohypophysis" is such that it can hardly have a common origin with the saccus vasculosus in the saccus infundibuli, but is probably derived from the diencephalic wall between the saccus infundibuli and the chiasma. This will mean, that the "neurohypophysis" in fish with the terminus of the tractus praeoptico-hypophyseus can be formed from slightly different parts of the hypothalamic floor, and these possibilities are indicated in fig. 138. It would then sometimes be formed by parts which are homologous with the eminentia region in higher vertebrates and sometimes by parts corresponding to the neural lobe.

Point 2 concerning the relations between the intermedia and the neurohypophysis in *Petromyzon* has not been introduced in this discussion. Although it strongly suggests homologies with higher vertebrates, it seems dangerous to pay too much attention to it because of the isolated systematic position of the group *Cyclostomata*. The innervation (point 3) is treated as a secondary criterium above. It is intimately connected with the function and can therefore hardly be regarded as equally significant as the early morphogenesis for homologizations. We must thus accept the fact that the tr. supraoptico-hypophyseus may have altered its terminus during the phylogenetic development.

CHAPTER XII

Some Functional Aspects on the Avian Neurohypophysis

Functional Differentiation

In the preceding chapter I have presented some evidence of the opinion that the *eminentia mediana* and the neural lobe in amniotes have been derived phylogenetically from a uniform organ, the walls of which consist of an inner ependyma, an intermediate fibre layer, and an external zone without coarse nerve fibres, the so-called glandular layer. Pituitaries of this simple type are present mainly among reptiles. In most birds the primitive pattern is usually confused by complications. The *eminentia* is characterized by the mighty development of the glandular layer, and the neural lobe proliferates in different ways. Cells tend to leave the ependymal layer as pituicytes and "glia cells" in the neural lobe and the *eminentia* resp. This structural differentiation of the neurohypophysis into two parts does not prove anything of a functional differentiation, however. It seems reasonable to assume that a compact neural lobe is a more effective incretory organ than a hollow sac and thus means a more effective and space-saving arrangement. A compact neural lobe with numerous internal vessels seems, at least, to contain more contact-spaces between the gland and the blood vessels than a hollow sac with capillaries only on the surface.

The innervation of the avian neurohypophysis distinctly shows, that a functional specialization is present. The neural lobe is the terminus of the tractus supraoptico-hypophyseus and contains lots of the specific neurosecretory substance of this tract. The deeper parts of the *eminentia* serve as a path-way for this tract, but the superficial parts of the *eminentia*, the so-called glandular layer, is undoubtedly a specific functional unit with very few connections with the supraoptico-hypophyseal tract and it is innervated mainly from the nucleus tuberis. It is very interesting to note that exactly the same observations were made in the cat by Nowakowski (1951).

The neurohypophysis in birds has been shown to contain the common neurohypophyseal principles: vasopressor, antidiuretic and oxytocic ones (cf. Heller's review 1950). In addition it is reasonable to assume that some substances capable of regulating the function of the adenohypophysis are produced in the *eminentia*

and transported to the epithelial part by the portal vessels (cf. p. 253). Is it possible to predict theoretically a relationship between the different endocrine principles and the functional differentiation revealed by the innervation?

Eminentia mediana

The fibre layer of the eminentia is of little interest in this connection since it belongs to the functional system of the neural lobe. The glandular layer has been called so in this book because of its probable glandular function, and some reasons for this opinion were presented (p. 197). Some complementary remarks will be made here on the basis of the preceding descriptions.

The glandular function of the eminentia mediana is proved mainly by its rich vascularization. It is hard to believe that the dense capillary net on its surface has a mainly nutritive function. Generally, the neural tube contains a very wide-spaced lattice of capillaries, and the few cells present in the relatively thin wall of the eminentia would reasonably require still fewer capillaries if the function of the vessels were mainly trophic. It could perhaps be suggested, also, that the ependymal cells are active in secreting substance into the ventricle, and that the material for this activity is supplied by the superficial capillary net via the vascular processes of the ependymal cells. The vacuoles along the ependymal surface in the ventricle support the opinion that something is secreted from the ependymal cells, but this support is not very strong because of the possibility of artificial formation of such vacuoles. On the other hand, if the dense net of capillaries serves to maintain the secretion into the ventricle only, it is impossible to understand the structure of more complicated eminentiae such as that in the goose (fig. 110 C). In this species a considerable number of cells in the eminentia have given up the contact with the internal surface, but attach to the external, vascularized wall in the same way as the ependymal cells. These emigrated glia cells cannot serve to establish a contact with the ventricle and would appear to be without functional meaning if the above hypothesis is true, but they are almost identical with the ependymal cells in their appearance and staining properties. The nerve fibres in the wall cannot require such a dense system of capillaries, since nerve fibres are known to have a very low metabolic rate.

Nowakowski (1951) suggests that the specific innervation of the eminentia in the cat is sensory and registers the content of adenohypophyseal hormones coming from the pars distalis in the portal vessels. The latter would, then, serve to bring the blood from the pars distalis into contact with the sensory organ in the eminentia. This view cannot be accepted, however, since the blood definitely flows *from the eminentia to the pars distalis* in birds and probably also in other amniotes and amphibians though decisive evidence is available only for some species (cf. chapter XIII).

The only reasonable interpretation of the density of the capillary plexus of the eminentia is that it serves to wash away secretory products and perhaps also to

supply the material for the secretory activity. The presence of stainable colloid in the glandular layer, its peculiar innervation and its intimate relation to the capillaries leave little doubt as to the origin of the secretions. Everything secreted into these superficial eminentia capillaries must necessarily be transported to the pars distalis by the portal vessels (chapter XIII). There are, thus, very favourable conditions for a transport of humoral transmitters of impulses from the nervous system in the glandular zone of the eminentia to the pars distalis. It is necessary, therefore, to examine the evidence of the presence of such humoral transmitters.

Convincing evidence for a transfer of hypothalamic impulses via the infundibulum to the adenohypophysis has been presented in experimental works with mammals, in which both the gonadotropic and the thyreotropic activity is controlled by the central nervous system in this way (e.g. Hillarp and Jacobsohn 1943, Brohlin 1945, Harris 1948, 1950, Hillarp 1949 a). A relation between the hypothalamus and the adenohypophysis is proved also for birds (Bissonette 1938, Benoit 1944, 1950, 1950 a, Wolfson 1941, 1945). Benoit and co-workers have, for instance, shown that light stimulation of eyes, pituitary, hypothalamus or adjacent ventral parts of the telencephalon causes an increased output of gonadotropic hormones in the duck, and this in turn makes the gonads grow rapidly. The experiments also show that a direct stimulation of the hypothalamus by the red fraction of the spectrum probably plays a rôle for the control of seasonal phenomena in the duck. Though the decisive proof of the route of the impulses from the hypothalamus to the pituitary is lacking the authors seem to be convinced that this route is direct, i.e. via the infundibulum. Anyway it has been shown that the regulation of the gonadotropic function is independent of the gonads. The reader is referred to Benoit's excellent review (1950, 1950 a).

The anatomical features of the avian pituitary leave only one way open for stimuli to reach the pars distalis from the hypothalamus, and this way is via the portal zone of the pars tuberalis from the eminentia mediana. The other sides of the pars distalis are surrounded either by the ossified wall of the sella, by the sinus cavernosus or by the diaphragma sellae (chapter XIV). The structure of these surrounding tissues is such that every nervous or vascular pathway would be observed. The contact between the pars distalis and the neural lobe is not very intimate in birds, and vessels or nerve fibres passing the separating membrane between these two parts have not been seen. The few fibres of the plexus caroticus which sometimes enter the pars distalis were discussed on p. 235, and it was concluded that they can hardly be concerned with a specific regulation of the glandular function. The impulses must thus necessarily be transferred via the eminentia mediana and the portal zone, and the key to the control of the pars distalis is thus the innervation of the glandular layer of the eminentia. *Since no nerve fibres have been seen leaving the eminentia but invariably turn back when they reach the surface, the impulses from the hypothalamus must be carried further by humoral transmitters, secreted into the capillaries of the eminentia.* The impulses would, then, be carried from the nuclei in the hypothalamus to the glandular layer of the

eminentia by the nerve fibres, and carried further via the portal vessels to the pars distalis by humoral transmitters. This idea of a combined nervous and humoral control of the pars distalis in higher vertebrates has been defended intensively by Harris and Green (cf. Green's review 1951, and Harris' review 1948).

If the above theory about the control of the pars distalis is accepted — and in fact the structural relationships in birds give a strong support to it — the functional differentiation of the glandular layer of the eminentia is to be regarded as a specialization for the control of adenohypophyseal functions. The nucleus tuberis, which is intimately connected with this layer, should thus be expected to be a centre for adenohypophyseal functions. However, it was briefly mentioned in chapter X that this nucleus is connected with a mighty system of non-medullated fibres which descend from praeoptic regions above the chiasma. There are thus good chances for anterior hypothalamic regions to influence the function of the pars distalis secondarily by way of the nucleus tuberis in agreement with Benoit's experimental results. Moreover, it is possible that some fine fibres in the tr. hypophyseus anterior enter the glandular layer, especially in the more rostral part. The exact course of this fibre system in the eminentia could not be stated with the same accuracy as that of the tr. supraoptico-hypophyseus, since it is not associated with any specific colloid.

Attempts have been made to localize the gonadotropic centres in mammals. Hillarp (1949 a) produced constant oestrus in rats by lesions in the post-optic part of the hypothalamus. His results give good evidence of a centre for gonadotropic (LH) functions localized in the hypothalamic area just ventral and anterior to the paraventricular nucleus. Small lesions further caudally on both sides of the ventricle have the same effect and indicate that a rather discrete tract passes from the centre to the eminentia mediana. It is difficult to state the exact morphological counterpart of this gonadotropic centre in birds, since the avian hypothalamus is much less differentiated. However, it may correspond to some nucleus situated in the region near the nucleus inferior hypothalami in birds, perhaps also to the rostralmost part of the nucleus tuberis. Hillarp's results are particularly interesting, for the animals with constant oestrus did not suffer from diabetes insipidus. This means that the gonadotropic centre and also the tract from this centre to the neurohypophysis are separate from the system regulating the antidiuretic hormone (supraoptico-hypophyseal system). In view of these results the statement of morphologically separate innervation systems connected with the neural lobe and the eminentia in the cat (Nowakowski 1951) and in birds (chapter X) is less puzzling.

It is of course also possible that some of the common posterior lobe principles are secreted from the eminentia, especially from its rostral parts. The present investigation showed, however, that the glandular layer of the eminentia has very few connections with the tractus supraoptico-hypophyseus and that the specific neurosecretory colloid accompanying this tract occurs only in the rostral parts of the glandular layer and only in small amounts. Now the tr. supraoptico-hypophyseus

has proved to be the final link in the system regulating the output of the anti-diuretic hormone in mammals (Fisher, Ingram, and Ranson 1938, Harris 1948, Hillarp 1949, Ortmann 1951). The presence of the very specific neurosecretory colloid along this tract both in mammals and birds may justify the opinion that it is concerned with the antidiuretic function also in birds. The glandular layer of the eminentia would, then, have very little to do with the antidiuretic hormone. Little can be said in this respect of the oxytocic and vasopressor activities.

Lobus nervosus

The *neural lobe* in birds has been shown to contain vasopressor, antidiuretic and oxytocic hormones, and also the specific "amphibian water balance principle" has been ascertained (Heller 1950). There has been much discussion about the mode of production of these hormones in mammals, and the contributions to this discussion offered by the avian material will be briefly surveyed below. The structural relations of the adenohipophysis in birds show that the neural lobe cannot possibly have to do with the control of the function of the adenohipophysis. The neurohypophysis and the adenohipophysis are usually separated by connective tissue, there are no nerve fibres from the neural lobe to the pars distalis, and the vascular bed of the neural lobe is drained directly into the sinus cavernosus without relation to the epithelial part. This is in agreement with experiments with mammals. Westman, Jacobsohn and Hillarp (1943) were able to show that the hypothalamic regulation of the gonadotropic activity in the rat is unchanged after extirpation of the neural lobe.

The Origin and Function of the Neurohypophyseal Colloid

A series of publications during the last few years have accumulated evidence of the origin of the Gomori-positive colloid in the supraoptic and paraventricular (resp. praeoptic) nucleus of mammals and fish (Bargmann 1949, Bargmann and Hild 1949, Ortmann 1951).¹ The original idea of a transport of colloid from this nucleus along the axones to the neurohypophysis (Scharrer and Scharrer 1940, 1944, 1945, Palay 1945, Hanström 1946, 1947) has thus been beautifully corroborated.

Some comparative considerations support this hypothesis. As mentioned in the preceding chapter my preparations of birds and reptiles which were treated according to Gomori (Bargmann 1949) show the presence of the specific blue-staining matter in the nerve cells of the supraoptic nucleus, in the proximal parts of the axones of these cells, and as more or less frequent granules along the tract to the neural lobe where large quantities of the substance are found. The presence of this substance within the supraoptic nerve cells strongly supports the opinion that it is formed there. The neural lobe of the sauropsids contains only ependymal cells and sometimes also free pituicytes and connective tissue elements in addition

¹ Unfortunately the following works could not be considered: Hild, 1950, 1950 a, Bargmann, Hild et al. 1950.

to the nerve fibres. It is hard to believe that the very specific Gomori-substance, which is present nowhere else in the brain, can be produced by cells which are so far different from the supraoptic nerve cells as are the ependymal cells and the pituicytes in the neural lobe. Bargmann (1949) and Ortmann (1951) have shown that the secretory cycle of the supraoptic nerve cells is accompanied by typical changes in the Nissl substance, and Nissl substance is not present in the cells of the neural lobe. A striking example in my collection is a section series of *Gambelia wislizenii*. The neural lobe of this lizard is of the primitive type with only ependymal cells in the neural lobe and no pituicytes. In spite of that, Gomori staining reveals the specific, blue-black colloid in the superficial parts of the thin wall of the neural lobe, and also in the nerve cells of the nucleus supraopticus (fig. 140).

Since no cells of any type similar to the colloid-producing supraoptic nerve cells are present in the neural lobe of sauropsids the only reasonable explanation is that the colloid is transported to the neural lobe from the supraoptic nucleus. This conclusion is, of course, strengthened by the fact that the colloid can be traced along the transport route in some animals.

Nowakowski (1951, p. 306) is also of the opinion that the Gomori-positive substance is secreted by the supraoptic neurons in the cat, but does not regard it necessary to assume a transport of this matter along the axones. He means that the synthesis may take place everywhere on the surface of the neurons. This hypothesis does not mean a great difference from the original one, however, and is hardly simpler. The synthesis would then take place without direct co-operation of the cell nucleus and be independent of the Nissl substance!

The conclusions above apply only to the Gomori-positive material and it is by far not certain that all interstitial material seen in an azan-stained avian neurohypophysis is of this kind. The glandular layer of the eminentia, at least, contains also a Gomori-negative colloid which is stained in azan.

Collin (1926) discussed the origin of the neurohypophyseal colloid in the duck and the fowl. He thought that it is formed in the pars distalis and is transported to the neurohypophysis in the lymphatic vessels of the pars distalis to the perivascular spaces of the neurohypophysis. In my material there is nothing to support this hypothesis. Lymph vessel in the pars distalis have not been observed. It is true that such spaces are difficult to find, but they would be readily distinguished if they contained stainable colloid. No direct transfer of colloid to the neural lobe, which contains most of the colloid, can occur in most specimens because of the connective tissue membrane between the lobes. The blood in the portal vessels can not carry the colloid to the eminentia for it has the opposite direction, and no colloid-filled lymph spaces have been observed in the portal zone. Moreover, the capillaries on the surface of the eminentia which are continuous with the portal vessels are characterized by the absence of Robin-Virschow's lymph spaces when they are sometimes embedded in the tissue of the eminentia. In most species of birds the intima piae of the eminentia is unbroken and smooth, and it is not penetrated by any openings whatever which could indicate the entrance of lymph vessels.

Since none of the structures required for a transport of colloid from the pars distalis to the neurohypophysis in birds has been seen, it seems necessary to abandon this theory. That some colloid in the mammalian neural lobe can be formed by invading intermedia cells seems probable (cf. e. g. Collin and Stutinsky 1949, Shanklin 1947, Romeis 1940, pp. 338 ff.).

Wislocki and King (1936) showed that the vessels of the pituitary region are permeable to colloidal dyes which are injected into the vascular system, and accumulation of the dyes can be stated in the neurohypophysis after the experiment. This exceptional permeability of the vessels opens the possibility that blood proteins behave in the same way, and that some colloidal matter in the neurohypophysis thus may have a haematogenic origin. This possibility is also mentioned by Gersh (1938, p. 435). Even if this origin is possible for some proteins in the neurohypophysis the hypothesis can hardly be applied to the Gomori-positive matter, since very strained explanations will then be necessary. Especially the presence of the Gomori-positive colloid along the supraoptic-hypophyseal tract and its restriction to the deeper, non-vascularized parts of the eminentia will then be difficult to explain.

Finally, it is also possible that some colloid is secreted by the cells in the neurohypophysis (ependyma, pituicytes, glia cells), but this can hardly be true for the Gomori-positive matter (cf. above). However, the tissue culture experiments which will be discussed below show with some degree of certainty that the pituicytes are capable of a secretory function.

The functional significance of the neurohypophyseal colloid in mammals has raised considerable interest in the last few years. The older opinions concerning the colloid accumulations usually called Herring bodies were critically reviewed by Gersh and Tarr (1935). They used the freezing-drying technique for neural lobes of *Canis*, *Felis*, *Macacus*, *Cavia*, *Bos*, *Sus*, and *Gallus*, a technique which is little liable to produce artefacts. They maintain that the "Herring bodies" are artefacts produced by the fluid fixatives, for no such bodies were present in the freezing-drying preparations. The results were criticized by Romeis (1940, p. 449) who presented evidence for the existence of some types of "Herring bodies" also in vivo, but his conclusions were mainly based on human material. In my preparations it can be distinctly seen that avian pituitaries fixed slowly and less adequately contain large granules and irregular bodies of stainable colloid, whereas specimens fixed by injection of the vascular system generally have the colloid more uniformly dispersed. Further, there is much less stainable colloid in the specimens fixed in alcohol-formalin-acetic acid than in those fixed in Bouin and Susa. This probably depends on a less complete precipitation in the former fixative, and it also opens the possibility that not all protein material is made insoluble by the freezing-drying process used by Gersh and Tarr, but that some components are washed away when the sections are treated with water. Although common preparations of avian pituitaries undoubtedly contain a number of "hyaline bodies" which are artefacts in agreement with Gersh and Tarr, these authors undoubtedly

go too far when they include also the nerve endings described by Tello (1912) and Bucy (1930) among the probable artefacts. These nerve endings are present also in birds (p. 228) and appear as "hyaline bodies" in common azan preparations.

Whereas previous authors in agreement with Herring (1915) regarded the hyaline material in the neural lobe as a hormone or a "precursor" of a hormone, Gersh and Tarr (1935) found that the solubility of this stainable colloid is different from that of the antidiuretic hormone. Unfortunately, it is uncertain whether their statements apply to the Gomori-positive colloid in the neural lobe, which has later been regarded as the antidiuretic hormone itself.

On the other hand, the regular association of the Gomori-positive colloid with the supraoptico-hypophyseal system, and the convincing experiments showing the importance of this system for the secretion of antidiuretic hormone (cf. Harris 1948) suggests a relationship between this hormone and the Gomori-positive colloid. Further, the detailed correlation between the production of this colloid and the water balance in rats shown experimentally by Ortmann (1951) is a very strong proof of the theory. The identical and unique staining reactions of the Gomori-positive colloid in mammals, birds, reptiles, and fishes justify the conclusion that it has the same function in all these animals, and Ortmann's results can therefore certainly be applied also to birds as was done in the preceding chapters.

The Functional Significance of Pituicytes, Glia Cells and Ependymal Cells

Bucy (1930) showed that the glious elements in the bovine pituitary differ morphologically from common oligodendroglia and microglia though these are stained by the same technique, and he introduced the term pituicytes. Gersh (1939) stated that some of these cells in mammals and birds ("parenchymatous glandular cells") differ from common glia cells in the presence of osmophilic granules. He also found certain variations in the granulation of these cells which seemed to coincide with fluctuations in the water balance of the animal, and he therefore concluded that the "parenchymatous glandular cells" secrete the antidiuretic hormone. Brooks and Gersh (1938, 1941) described basket-like fibre structures in the neurohypophysis of the rat, which were interpreted as specific secretory nerve endings on the surface of the probable gland cells. Similar structures were also described by Stutinsky (1949) in the dog but were said to be associated with degenerating cells, and when observed in man these nets were regarded independent of parenchymatic cells (Romeis 1940, p. 441). In birds, especially in the goose, such basket-like formations are frequent but are not associated with any cells but are independent neurofibrillar structures (fig. 132). The combination of the Bodian impregnation with azan staining proved especially favourable for deciding the relations between cells and nerve fibres, whereas this is difficult in Cajal preparations. Since Brooks and Gersh used the latter method their conclusions do not appear to be definite. Consequently, the innervation pattern hardly supports the

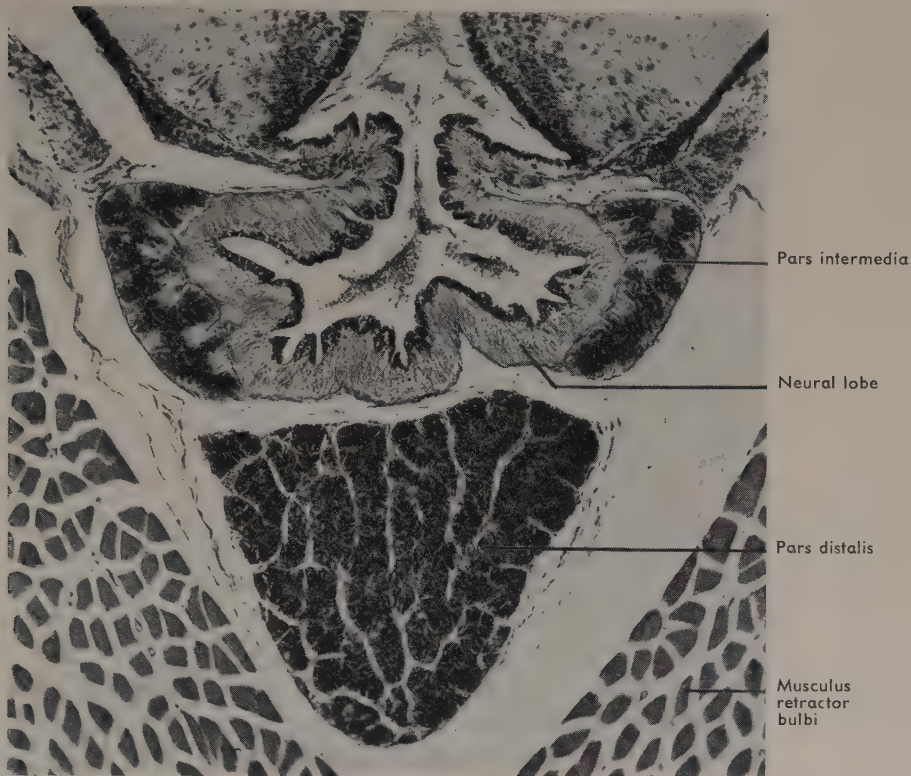


Fig. 140. *Dipsosaurus dorsalis* (lizard of the family Iguanidae). Horizontal section of the pituitary. The nuclei are entirely restricted to the ependyma along the recessus in the neural lobe. — Bouin, paraffin 12μ , haematoxylin of Ehrlich and eosin. $100\times$.

theory of a secretory activity in the pituicytes. Also the significance of the osmophilic granules has been critically commented (Hickey, Hare and Hare 1941).

Griffiths (1938) found pituicytes in all vertebrate classes from fish to mammals and concluded after a review of the literature that the vasopressor and probably also other neurohypophyseal principles are secreted by them. Tissue cultures of pituicytes indicate that a secretion of the vasopressor principle takes place from these cells also in the absence of nerve fibres (Geiling and Lewis 1935, Anderson and Haymaker 1935, Lewis 1938). Since the results obtained by tissue cultures appear to be conclusive, it may be regarded as highly probable that pituicytes are able to secrete the vasopressor substance. No definite result concerning the antidiuretic or oxytocic principles was obtained with these tissue cultures (cf. Anderson and Haymaker 1935, Lewis 1938, p. 465). Thus, the statement that the antidiuretic hormone is identical with or, at least, associated with the Gomori-positive colloid and that it comes from the supraoptic nucleus is not contradicted by the tissue culture experiments.

In view of the fact that vasopressor substance can be elaborated in the pituicytes it is not astonishing that these cells, and also ependymal cells and glia cells in the eminentia of birds, stain in a way slightly different from common glia cells. Whereas common glia cells require special methods the cells of the neurohypophysis including their processes are clearly visible also in Bodian-azan preparations if the staining is perfect (p. 199). Comparative studies of the material of birds and reptiles in my collection show that ependymal cells and pituicytes in the neural lobe are functionally equal. Closely related species, e.g. *Perdix* and *Phasianus*, illustrate this clearly. In the former species we find practically only pituicytes in the compact neural lobe, whereas the latter has a hollow neural lobe with almost exclusively ependymal cells. It is hard to believe that closely related species as the two mentioned here differ essentially in the hormone production of their neural lobes, the more so as the same neurohypophyseal principles have been found in all vertebrates from mammals to teleosts (Heller 1950). It must therefore be concluded that the function of the pituicytes can be exerted by cells lining the ventricle just like common ependymal cells. Similar examples are found in numerous lacertilia in which the neural lobe may lack pituicytes completely (fig. 140).

The result of the present investigation is thus slightly different from Griffiths' (1938) statement that pituicytes are found in all vertebrates from fishes to mammals. Griffiths means by pituicytes the same as Bucy (1930), i. e. free cells in the neural lobe without relation to the ventricle. If defined in this way the pituicytes are definitely absent or very rare in some neural lobes of birds and lacertilians as well as in *Sphenodon* (cf. p. 239). Since the ependymal cells and the pituicytes as shown above are closely related functionally the difference between Griffiths' opinion and mine is reduced to a question of nomenclature, however.

The cells in the eminentia mediana substitute each other in the same way as the cells in the neural lobe. In many birds there are practically only ependymal cells in the eminentia, but in others there are also numerous free "glia cells" in the wall. The functional identity of the ependymal cells and the free cells in the eminentia is suggested also by the identical staining reaction and the characteristic mode of attachment to the external wall (fig. 110). Thus, if the glia cells in the eminentia have a specific function, this function can most probably be exerted also by the ependymal cells.

Further, the staining reaction of ependymal cells and glia cells in the eminentia is identical with that of pituicytes and ependymal cells in the neural lobe. Tinctorial differences thus do not indicate a functional difference between the cells of the neural lobe and the eminentia, and the structural differences can hardly be regarded as significant. However, since definite proof is lacking, I have treated the free cells in the neural lobe and in the median eminence under different names, in order to avoid confusion.

CHAPTER XIII

The Vascular System of the Avian Pituitary

Literature

An extensive survey of the pituitary vessels in vertebrates has recently been published by Green (1951), who also gives a brief description of the vessels in the avian pituitary. Green found a broad anastomosis between the carotids behind the pituitary. The eminentia is covered by a dense capillary net, supplied by superior hypophyseal arteries. The eminentia plexus is drained by portal vessels which via the pars tuberalis pass to a secondary capillary plexus in the pars distalis. The neural lobe is supplied by arteries which are separate from those to the eminentia. The capillaries of the neural lobe form a superficial net from which regular arcades project inwards to the neighbourhood of the recessus infundibuli. The pituitary is drained directly into the surrounding venous sinuses. The present investigation corroborates most of Green's statements and may add a few further facts. According to Romeis (1940, p. 500) and Corneliac (1935) the hypophyseal circulation in *Columba* and *Gallus* is described also by David (1932). Since the Jassy school has accepted the idea that the blood flows from the pars distalis to the eminentia in the portal vessels it seems highly probable that David's opinions differ fundamentally from those in the present book. Unfortunately, it has not been possible to get David's paper and it can therefore not be commented on in detail.

Apart from the said works practically nothing has been published on the hypophyseal circulation in birds. It is interesting from an historical point of view that the portal vessels were observed and drawn already by Müller (1871), who called them "Arterienzweige" and thought that they supply the pars distalis. As pointed out by Green the portal vessels have been drawn also by other investigators (Economo 1899, Krause 1922, Rahn 1939, Drager 1945), but their significance was not understood.

It should be mentioned here that the vessels labelled "hypophyseal arteries" in the works on the pigeon pituitary by Schooley (1937, fig. 3) and Schooley and Riddle (1938) are the internal ophthalmic arteries and thus have nothing to do with the pituitary.

Descriptions of the arterial system in the head of birds are fairly numerous (e.g. Hahn 1830, Gadow 1891, Hofmann 1900, Beddard 1905, Neumann 1914, Hafferl

1921, 1933, Kappers 1933, Corneliac 1935, Rost 1939), but the supply of the pituitary has not been described by these authors. The embryonic history of the head arteries in birds is dealt with by Kastschenko (1887), Twining (1906), Sabin (1917), Hafferl (1921) and Hughes (1934). The head veins are briefly described by Corneliac (1935), but apart from this work they have hardly been studied in adult birds since Neugebauer published his excellent monograph in 1845. The embryonic history of the head veins was dealt with by v. Gelderen (1924, 1924 b, 1925, 1933), Sabin (1917) and especially by Hughes (1934).

In general I have tried to follow the terminology of Hofmann (1900) and Hafferl (1921) for arteries and that of Neugebauer (1845) for veins. Hughes' work is informative and clear, but the terms are not international, and are generally derived from those of the said authors.

Arterial System of the Pituitary Region

The arteries of the pituitary region are all branches of the arteria carotis interna (Hafferl 1921 = a. carotis cerebralis, Hofmann 1900). The proximal parts of the a. carotis externa are reduced in the adult, and only the end branches within the head persist. They receive their blood from a secondary anastomosis with the internal carotid just behind the head (cf. Hafferl 1921, Hughes 1934). Laterally of the foramen magnum the carotis interna emits an a. occipitalis and an a. ophthalmica externa (= a. stapedia, Hafferl) and enters the canalis caroticus in the cranial base near the foramen nervi facialis (syn. Falloppii, cf. Marinelli 1936). Surrounded by the vena carotis (Neugebauer) the a. carotis interna then runs rostro-medially near the external surface of the bone, medially of the cavum tympani and dorsally of the tuba Eustachii to the base of the rostrum sphenoidale. From here the carotis turns abruptly dorso-medially and meets its contralateral partner in the posterior part of the sella turcica. Near the base of the rostrum sphenoidale each carotis emits first an a. palatina (syn. Vidiana) along the ramus palatinus of the nervus VII, and immediately after that an a. spheno-maxillaris which is accompanied by the main trunk of the autonomic nerve which follows the carotis from its entrance into the cranium (fig. 141, 143).

This general description applies to most birds in my collection, but some variation has been noted. In *Diomedea epomophora* the carotis does not enter the cranium until it reaches the base of the rostrum sphenoidale, and it emits the a. Vidiana and a. spheno-maxillaris when it is still outside the cranium. It runs in a furrow in the bone from the occipital region to its point of entrance. In *Procellaria* the channel is also incompletely closed posteriorly, but in *Pelecanoides* there is a complete tube as in other birds. Osteological observations (Marinelli 1936) indicate that the carotis runs outside the cranium to the rostrum also in *Sula* and *Phalacrocorax*.

The a. spheno-maxillaris and a. Vidiana are highly variable in birds. Thus, in the majority of birds there are two arteries from the carotis cerebralis, accompanied

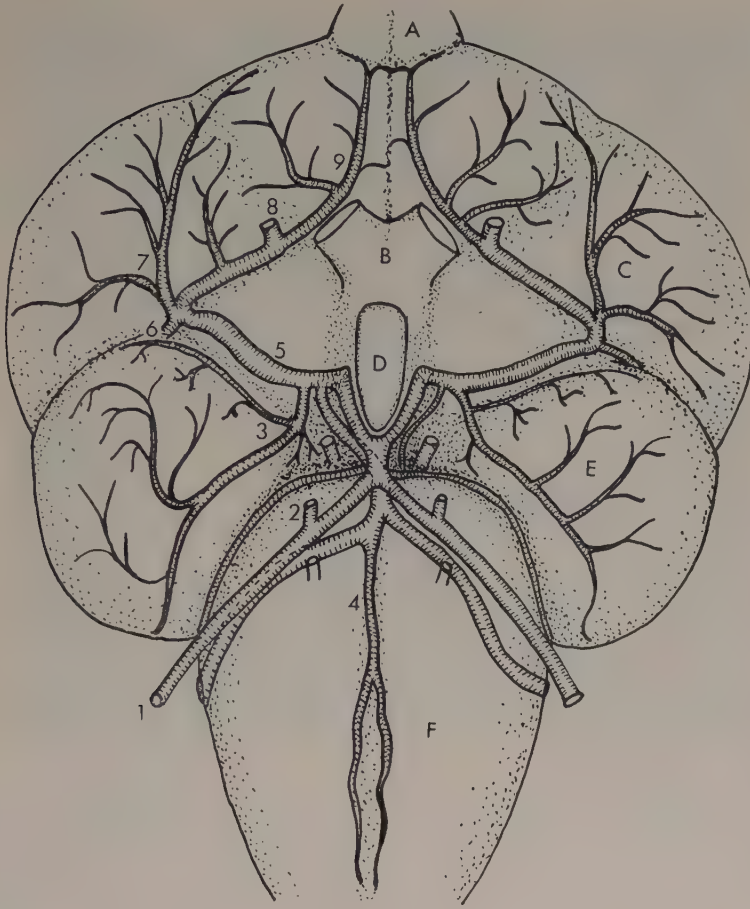


Fig. 141. Ventral view of the brain of *Columba livia*, showing the distribution of the carotis interna.

A = lobus olfactorius, B = chiasma opticum, C = left hemisphere, D = pars distalis of the pituitary, E = tectum opticum, F = medulla oblongata.

1 = Carotis interna, 2 = arteria spheno-maxillaris + Vidiana, 3 = artery to the tectum (bigeminal artery, Hughes 1934), 4 = a. basilaris, 5 = ramus anterior of the carotis, 6 = a. cerebri posterior, 7 = a. cerebri media, 8 = a. ethmoidalis, 9 = a. cerebri anterior.

Drawn from a cleared specimen, injected with starch-cinnabar and partly dissected. 6X.

by the autonomic carotis nerve (a. spheno-max.) and the ramus palatinus VII (a. Vidiana) resp. In many birds (*Regulus*, *Parus*, *Emberiza*, *Numenius*, *Enicognathus*, *Spheniscus*) the two arteries use different foramina in the sphenoid bone, but in other birds (*Pelecanoides*, *Anser*, *Gallus*, *Xolmis*, *Leptastenthura*) both arteries use the same foramen, and in *Diomedea* and *Procellaria* they are emitted when the carotis is still outside the bone. In *Columba* and *Sephanoides* both nerves accompany one and the same artery through a channel in the bone, and this artery gives rise to two branches which probably correspond to the a. spheno-

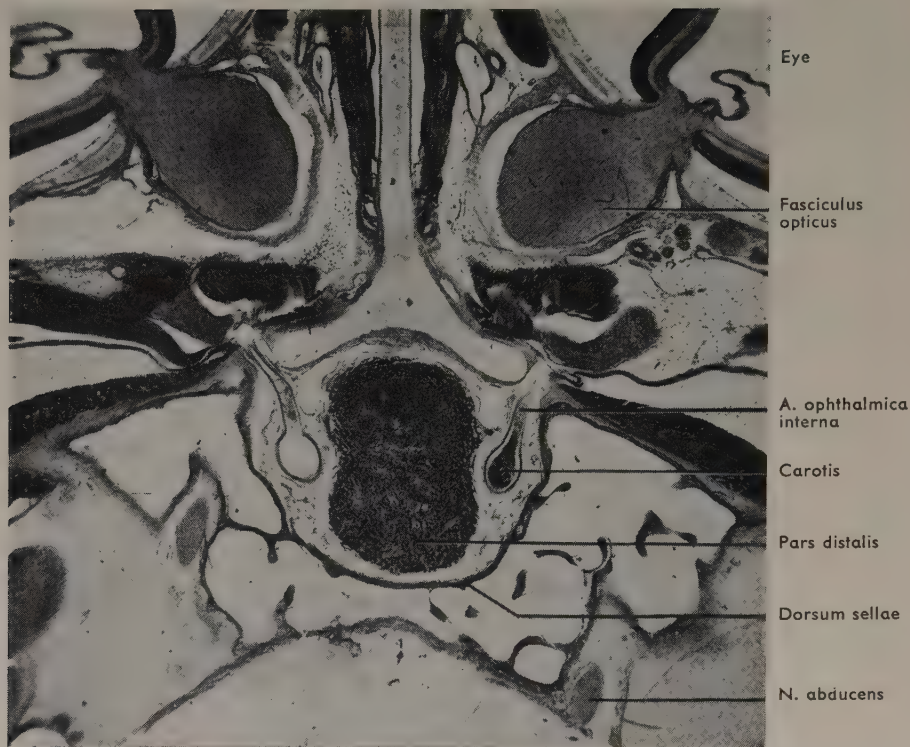


Fig. 142. *Gallus gallus*, juv., 1.5 days after hatching. Horizontal section through the pituitary, showing the a. ophthalmica interna. The vena ophthalmica interna which accompanies the artery is not very distinct, since it is empty, nor is the sinus cavernosus between the pars distalis and the sella. — Bouin, celloidin 40 μ , phosphotungstic acid haematoxylin. 22 $\times$.

maxillaris and a. Vidiana resp. In the latter case the basal parts of the arteries seem to have fused.

Immediately after the said arteries have been emitted the carotids bend dorso-medially and enter the posterior end of the sella turcica, and here an intercarotic anastomosis is formed. This anastomosis has the shape of a long, transversal vessel in the passeriform birds, and also in *Picus*, *Enicognathus*, *Caprimulgus*, *Gallus*, *Sephanoides*, and *Apus* (fig. 143, 153). In *Columba*, *Melanitta*, *Anser*, *Larus*, *Strix*, *Diomedea*, *Priocella* and *Procellaria* the two carotids have fused into a longitudinal vessel for a considerable distance (fig. 141), and in *Pelecanoides* and *Milvago* there is a shorter longitudinal anastomosis. In the specimens of *Leptastenthura* and *Elaenia* there is no communication between the carotids behind the pituitary, and since the anastomosis is exceedingly small also in *Xolmis* this may be characteristic of the primitive Passeres (Mesomyodes).

From the anastomosis or just caudally of it the carotis emits one or a few small arteries which in adult birds mainly supply the immediate surroundings, and in

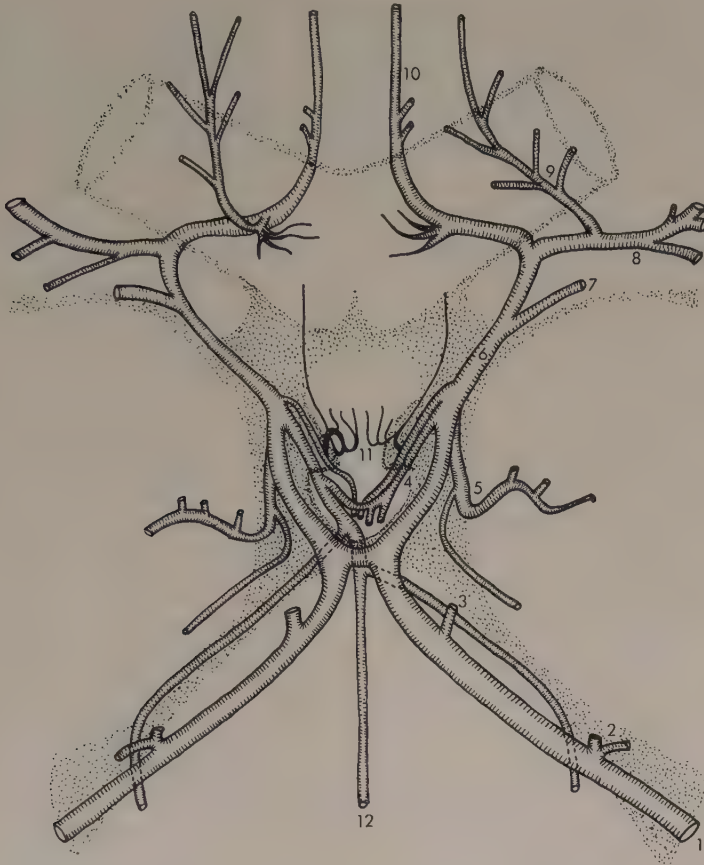


Fig. 143. *Pica pica*, ♂. Ventral view of the arteries of the brain base. Confer fig. 141.

1 = arteria carotis interna, 2 = a. Vidiana, 3 = a. sphenomaxillaris, 4 = ramus posterior of the carotis, 5 = artery to the tectum, 6 = ramus anterior of the carotis, 7 = a. cerebri posterior, 8 = a. cerebri media, 9 = a. cerebri anterior, 10 = a. ethmoidalis (on each side of the septum interorbitale in the orbit), 11 = arteriae infundibulares (compact black), 12 = a. basilaris.

Specimen, injected with starch-cinnober, decalcified and cleared. 8.5×.

some species an *a. hypophyseal inferior* branches off to the neural lobe. This will be discussed below.

After the carotids have separated again they pass rostrally along the sides of the pituitary, but make a sharp bend before they reach the rostral end of the organ and pass through the diaphragma sellae into the brain cavity. The intrasellar part of the carotis emits the true *a. ophthalmica interna*, just before it turns dorsally (fig. 142, 147). Embryological evidence indicates that this vessel corresponds to the *a. ophthalmica interna* of mammals (Hafferl 1921, Hughes 1934). In some species in my material this artery is present also in the adult whereas it is completely

reduced in other forms. It is interesting to note that the absence of the a. ophthalmica seems to be typical of the passeriform birds and their relatives (*Sephanoides*, *Apus*, *Dryobates*, *Picus*, *Cuculus*), whereas it is present in all other birds in which the arteries could be studied (all Anseriformes, Procellariiformes, *Columba*, *Spheniscus*, *Milvago*, *Numenius*, *Tringa*, *Enicognathus*, *Larus*, *Gallus*, *Lyrurus*). This feature thus seems to be of a certain taxonomical importance. When present the a. ophthalmica interna runs along the sides of the pituitary rostrally and enters the orbit by a foramen in the sphenoid which is separated from the foramen opticum by a thin osseous lamella. I have never seen any branches to the pituitary from this internal ophthalmic artery. Either it does not branch at all within the sella, or it emits a few small arterioles to the perist of the sella and to other connective tissue membranes.

When the carotis cerebialis touches the diencephalic wall it divides into two main stems: the *ramus posterior* and the *ramus anterior* (Hafferl 1933). Inside the brain cavity proper, between the diaphragma sellae and the point of division, the carotids emit one or a few vessels on each side, which are the main arteries of the hypophyseal region. They will be called *arteriae infundibulares* and will be treated in detail below (fig. 143, 147, 149).

The rami posteriores of the carotids are, as pointed out by Beddard (1905), distinctly asymmetrical in most birds. Both give off a branch to the tectum opticum, but this branch may also arise independently from the carotis. Further some branches from the posterior rami disappear in the furrow between the medulla oblongata and the diencephalon. As a rule only one of the posterior branches gives rise to the a. basilaris in the midline of the medulla oblongata. In *Leptastenthura* and *Elaenia* both posterior rami are of the same size and contribute both to the a. basilaris, but in the other birds (e. g. *Corvus* and *Pica*) in which both sides contribute to the basilar artery the rami posteriores are of very different size so there is still a striking asymmetry. One of the rami posteriores is then hardly more than a capillary. It is interesting to note that the ramus posterior giving rise to the a. basilaris is either right-hand or left-hand and that opposite conditions may be found in different specimens of the same species (table 4). This alternating asymmetry in relation to left and right may seem remarkable and reminds of the asymmetrical pituitary of snakes, which sometimes has a left-hand neural lobe and sometimes a right-hand one, as will be shown in another paper.

The asymmetry of the root of the basilar artery in birds may, however, depend on the exceptional haemodynamic conditions caused by the nearby intercarotic anastomosis. If one of the two ways from the intercarotic anastomosis to the a. basilaris offers less resistance to the blood, more blood will choose this way. Now it seems probable that an acceleration of the blood stream through a vessel causes an increase of the diameter (cf. review in Hughes 1934), and the pre-existing small difference between the two rami posteriores will thus rapidly be multiplied. It is interesting to note, in this connection, that both roots persist in the specimens of *Leptastenthura* and *Elaenia* which lack intercarotic anastomosis. The

Table 4

The variations in the supply of the arteria basilaris from the rami posteriores of the carotids. The table indicates in how many specimens the a. basilaris arises from the right and left ramus resp. and in how many specimens the vessels are symmetrical. Occasional minute anastomotic vessels between the ramus posterior and the basilar artery are not considered.

Species	Right ramus	Left ramus	Both rami
<i>Diomedea epomophora</i>	1	—	—
<i>Priocella antarctica</i>	1	—	—
<i>Procellaria aequinoctialis</i>	1	—	—
<i>Pelecanoides magellani</i>	—	1	—
<i>Anser indicus</i>	—	1	—
<i>Melanitta nigra</i>	—	1	—
<i>Larus argentatus</i>	1	1	—
<i>Numenius phaeopus</i>	1	—	—
<i>Tringa totanus</i>	—	1	—
<i>Gallus gallus</i>	1	2	—
<i>Columba livia</i>	2	2	—
<i>Milvago chimango</i>	1	—	—
<i>Strix aluco</i>	—	1	—
<i>Enicognathus leptorhynchus</i>	—	1	—
<i>Cuculus canorus</i>	—	1	—
<i>Picus viridis</i>	1	—	—
<i>Caprimulgus europaeus</i>	—	1	—
<i>Apus apus</i>	1	2	—
<i>Sephanoides sephanoides</i>	—	1	—
<i>Leptastenthura aegith.</i>	—	—	1
<i>Elaenia albiceps</i>	—	—	1
<i>Regulus regulus</i>	3	2	—
<i>Parus atricapillus</i>	1	2	—
<i>Riparia riparia</i>	1	—	—
<i>Emberiza citrinella</i>	1	1	—
<i>Passer montanus</i>	1	—	—
<i>Pica pica</i>	1	2	—
	19	23	2

asymmetry in the vessels around the avian pituitary has, thus, no relation to the asymmetry in the pituitary of snakes.

The ramus anterior of the carotis cerebralis gives rise to the three usual branches to the brain (a. cerebri posterior, media and anterior, cf. Beddard 1905, Kappers 1933) and leaves the brain case into the orbit dorsally of the opticus as an arteria ethmoidalis on each side (Hofmann 1900, Hafferl 1933, Corneliac 1935). The a. ethmoidalis may also carry blood into the brain from the orbital arteries in some species (fig. 14I, 143).

The "circle of Willis" in birds is incomplete caudally, for the rami posteriores of the carotis usually do not fuse. The circle is incomplete also rostrally in most birds,

for the anterior cerebral arteries do not communicate or the anastomotic vessel has capillary dimensions. Kappers (1933) recorded a distinct anastomosis between the anterior cerebral arteries in *Vultur* and *Antigone*, but Beddard (1905) could not find it in the 16 bird species examined by him. In one injected pigeon in my collection there is such an anastomosis hardly broader than a capillary, but I have searched for it in vain in other specimens. The lateral parts of Willis' circle are, however, distinct also in birds and can certainly be homologized with the lateral parts of the circle in mammals. This is important for this part of the arterial system is intimately related to the hypophyseal supply.

Venous System of the Pituitary Region

Neugebauer's description from 1845 is still the most complete account of the venous system of the brain base in birds. In *Meleagris* Neugebauer found an "annulus venosus basilaris" which is said to surround the chiasma and the hypophysis. This annulus is said to be connected with and to receive blood from the ventral surface of the hemispheres (vv. basilares cerebri longitudinales media et laterales), from the sinus sphenoidi situated between the tectum and the hemisphere on each side, from the sinus petrosi between the tectum and the medulla, and from the unpaired vena basilaris medullae oblongatae. The communication with the extracranial veins are said to be established by three pairs of vessels from the annulus: Two pairs connect the annulus with the v. ophthalmotemporalis, one pair between the optic and olfactory foramina, and one pair ventrally of the opticus, and the third outlet is the vena carotis along the a. carotis cerebialis. After Neugebauer only Corneliac (1935) has published an original investigation of these veins in birds, viz. in *Columba*, *Gallus*, *Anas* and *Anser*. He refound Neugebauer's vena carotis in the canalis caroticus, and stated that it fuses with that of the other side to form a plexus of veins in the sella turcica around the pituitary. He does not pay much attention to intracranial connections of this plexus, but states that it is connected with the orbital veins by a v. ophthalmica along the a. ophthalmica interna.

Hughes (1934) did not follow the development of the veins to the adult stage in *Gallus*, but recognized the development of the perihypophyseal plexus of veins and that of the vena carotis and found that they develop secondarily without connection with the primary head veins. Hughes also describes a connection between the veins of the sella and the orbital veins.

My own study has been focussed on the venous vessels in the sella and their connections, since they are especially important to the function of the pituitary. I start the descriptions with the large venous caverns in the sella, which will be called "sinus cavernosus". The term is used because it describes the structure well — there is in fact a cavernous sinus — and it is not regarded as a comparative anatomical term. In fact, the sinus cavernosus of birds is not homologous with that of mammals (see p. 294).

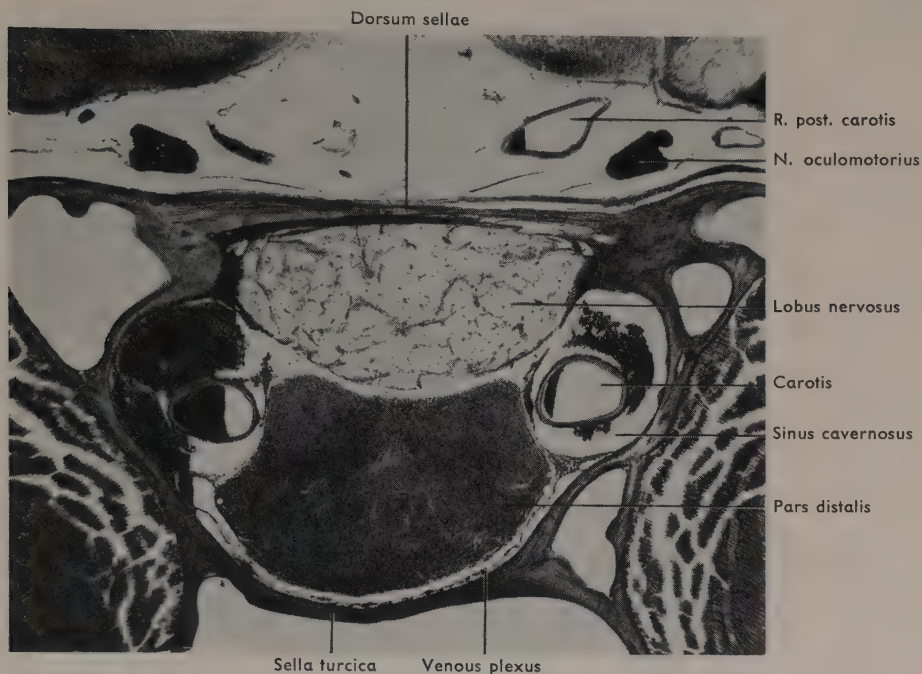


Fig. 144. *Priocella antarctica*. Transversal section through the sphenoid region with the pituitary in situ. Note that the sinus cavernosus is represented by a delicate plexus along the ventral side of the pars distalis. — Bouin, celloidin 60μ , phosphotungstic acid haematoxylin of Mallory. $20\times$.

Sinus cavernosus occupies a considerable part of the sella in birds. It surrounds the carotids and the carotid anastomosis in the caudal parts. Further rostrally it has its greatest extension along the sides of the pituitary, whereas the dorsal and ventral surfaces of the latter partly attach directly to the diaphragma and the dorsum sellae and to the periost on the floor of the sella resp. Smaller venous channels are, however, present everywhere around the pars distalis and the neural lobe (fig. 2—5, 144). In all small passerine birds and in *Apus*, *Sephanoides*, *Cuculus*, *Oceanites* and *Columba* the sinus cavernosus consists of a simple cavern which surrounds the anastomosis and the pituitary (fig. 160). In larger species the sinus is generally more complicated. Thus, for instance, it is partly subdivided by membranes and is traversed by strings of connective tissue in *Enicognathus*, *Tringa*, *Caprimulgus*, *Strix*, *Pelecanoides* and *Pica*, and in the largest birds it is subdivided into a dense net of wide and anastomosing channels (*Diomedea*, *Priocella*, *Procellaria*, *Gallus*, *Melanitta*, *Anser*, *Larus*) (fig. 145).

The walls of the sinus cavernosus are very simple. They are formed mainly by the surrounding connective tissue structures, i. e. the periost of the sella, the capsule of the pituitary, the connective tissue septa and the adventitia of the carotis. The only trace of a specific vascular wall is a thin endothelium, but even

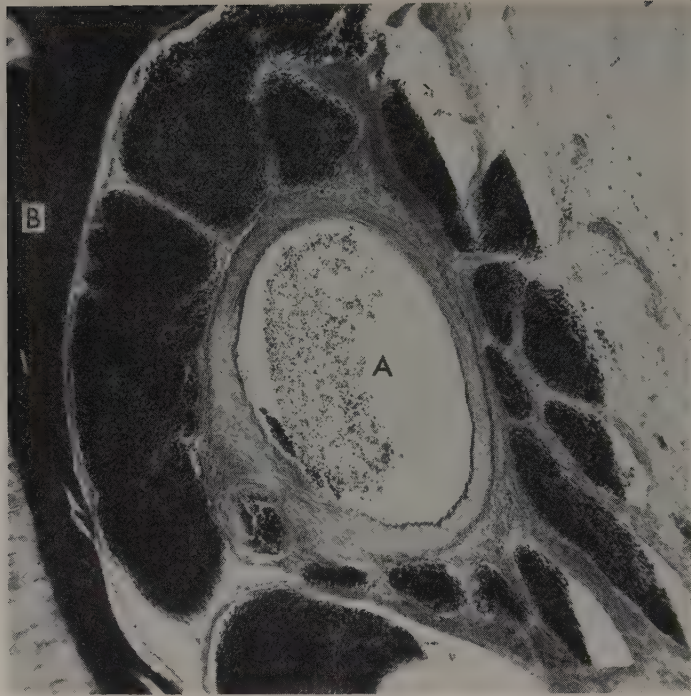


Fig. 145. *Procellaria aequinoctialis*, transversal section. The photograph shows the complicated sinus cavernosus around the carotid (A), near the point where the latter leaves the sella. B = osseous wall of the sella. — Bouin, celloidin 40 μ , phosphotungstic acid haematoxylin of Mallory. 75 $\times$.

this is difficult to distinguish in some places. The walls of the sinus cavernosus often contain small lymphatic nodules, and a peculiar type of epitheloid proliferation of the walls has been observed in some cases. These epitheloid cells have almost the appearance of glandular cells, but must have been formed by mesenchymatic elements in the walls.

The *venae carotides* (Neugebauer 1845) are the most important outlet of the sinus cavernosus in birds. They form the direct continuation of the sinus cavernosus caudally or ventro-caudally. The latter surrounds the anastomotic part of the aa. carotides completely so this part of the arteries lies in the middle of a large vein. Further caudally the sinus cavernosus divides into the two *venae carotides* which run along and surround the *arteriae carotides* more or less completely within the bone. The *arteriae sphenomaxillaris* and *Vidiana* are usually also accompanied by small veins which are continuous with the v. carotis. The latter carries the blood to the occipital region where it is finally transported to the *vena jugularis interna* (cf. Neugebauer 1845, Corneliac 1935).

The connections between the sinus cavernosus and the orbit are far more complicated than the scattered literature records indicate. These connections are

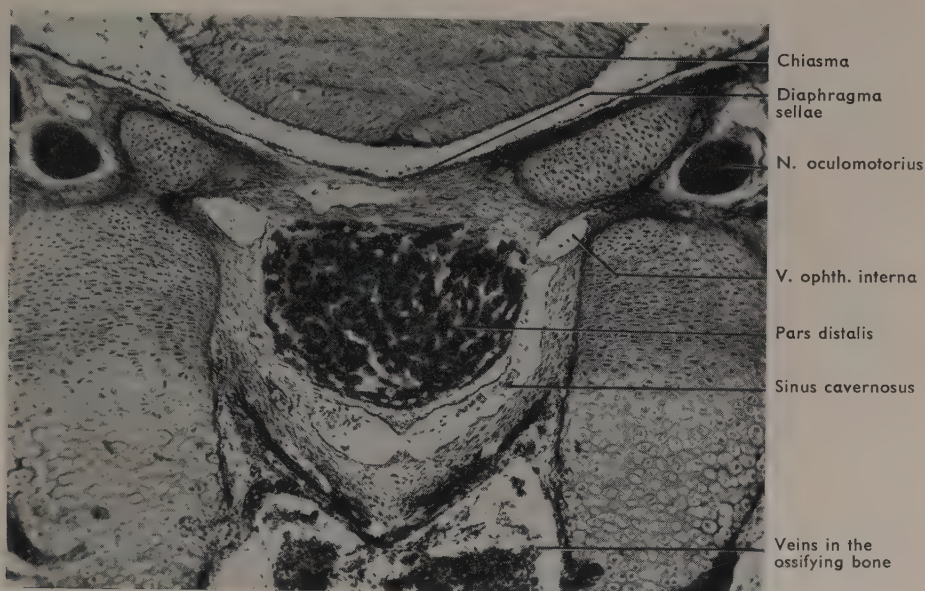


Fig. 146. *Anthus t. trivialis* (L.), hatching young. Transversal section through the sphenoid region showing the origin of the venae ophthalmicae internae in the sella. This vein is distinct in the specimen in spite of the fact that the corresponding artery has been completely reduced. Note the foramen for the vein in the cartilage. — Bouin, paraffin 8 μ , azan, 90 $\times$.

invariably present in my specimens, as far as the preparations show the vessels clearly, but they are highly variable. In the pigeons in my collection there are two pairs of such veins. One pair accompanies the a. ophthalmica interna and will therefore be called *v. ophthalmica interna* (fig. 146). It arrives into the orbit together with the artery through the foramen just ventrally of the foramen opticum and may be present also in birds which lack the a. ophthalmica interna. Further, the sinus cavernosus extends rostro-laterally up to the oculomotorius nerve, which runs along the margin of the sella, and emits a vein along this nerve to the orbit. This vein will be called *vena nervi III*, and enters the orbit together with the nerve by a foramen which is continuous with the foramen opticum in the five pigeons (2 section series and 3 skulls) in which this could be seen. Both these veins are present in the majority of birds, but the inter- and intraspecific variations seem to be very large. Thus, in two specimens of *Pica pica* there are two distinct veins, whereas a third specimen only has the v. nervi III. Two distinct veins though sometimes of different size were present in specimens of *Gallus*, *Lyrurus*, *Dendrocopos*, *Regulus*, *Parus*, *Riparia*, *Caprimulgus*, *Sephanoides*, *Melanitta*, *Anser*, *Larus*, *Numenius* and *Spheniscus*, whereas the v. ophthalmica interna was the only vein in *Milvago*, and the v. nervi III formed the only connection with the orbit in *Picus* and the said specimen of *Pica*. In *Pelecanoides*, *Diomedea* and *Priocella* both veins are vestigial though present, but in one specimen of *Procellaria* there is only

a large v. ophthalmica interna. In all birds except the pigeons the foramen for the n. oculomotorius and the v. nervi III is separated from the foramen opticum.

The dorso-lateral extension of the sinus cavernosus giving rise to the v. nervi III was followed in a number of cases upwards along the sides of the chiasma on each side. This perichiasmatic ring seems to correspond to the anterior part of the annulus venosus basilaris of Neugebauer, but I was not able to state whether it emits another orbital vein dorsally of the chiasma, because that region had usually been trimmed away.

Neugebauer and Corneliac are of the opinion that the veins between the orbit and the sella carry blood to the sella, and the calibre of the different vessels in fact supports this opinion. In any case the stream in the v. carotis must be directed caudally and the pituitary is thus situated in a large stream of venous blood which moves caudally and almost directly arrives into the jugular veins and consequently into the main circulation.

Connections with cerebral vessels. Neugebauer (1845) describes extensive communication between the annulus venosus basilaris and the cerebral veins in *Meleagris*. Corneliac (1935, fig. 15) has figured the sinus petrosus superior in continuity with the sinus cavernosus, but does not describe any more cerebral connections of the sinus in pigeon and fowl. Van Gelderen (1933) is of the opinion that almost all blood from the brain sinuses in birds leaves the cranium in the occipital region via the vv. occipitales, or via the vv. occipitales emissariae. The system of ophthalmic veins—sinus cavernosus—vv. carotides would then be independent of the cerebral circulation. In my preparations it is very difficult to find any connections with the cerebral circulation. In the pigeons, for instance, there is a variable connection through the dorsum sellae with the vena basilaris medullae oblongatae, but the vessel is very small and definitely absent in most other birds. The connection with the sinus petrosus has hardly more than capillary dimensions and could not always be discerned. Only the rostral prolongations of the sinus along the sides of the chiasma are of such a size in some birds (*Gallus*, *Lyrurus*) that they may carry greater quantities of blood from the ventral surface of the hemispheres to the region of the sella. Summing up, then, it appears that the cerebral circulation has very little to do with the veins of the pituitary region.

The Supply of the Pituitary Proper

The short communication in Green's paper (1951) on the vessels of the avian pituitary has, in general, been corroborated by the present investigation. In short, the arrangement may be characterized as follows (fig. 147): 1) The neural lobe receives an independent blood supply from arteries which in some birds are branches of the infundibular arteries, but in other birds are true inferior hypophyseal arteries coming from the intercarotic anastomosis. The neural lobe is drained directly into the sinus cavernosus. 2) The dense capillary net of the eminentia (primary plexus) is supplied by the infundibular arteries. It is drained



Fig. 147. Diagram of the vessels of the pituitary of *Columba livia*.

A = chiasma opticum, B = diencephalon, C = medulla oblongata, D = dorsum sellae, E = osseous floor of the sella.

1 = arteria carotis interna, 2 = a. hypophyseae inferior, 3 = anterior ramus, and 4 = posterior ramus of the a. carotis, 5 = a. infundibularis, 6 = primary capillary plexus on the eminentia, 7 = portal vessels, 8 = secondary plexus in the pars distalis, 9 = capillary bed of the neural lobe, 10 = a. ophthalmica interna.

From a thick median section through the sphenoid region of a pigeon injected with ink and cleared in benzyl benzoate. The capillary nets are simplified, and the perihypophyseal veins are not considered.

by the portal vessels which pass along the portal zone of the pars tuberalis to the "secondary plexus" in the pars distalis. The latter part is drained directly into the sinus cavernosus. The different parts of this vascular pattern will be treated in detail below, since, in my opinion, they are highly important for a correct understanding of the function of the pituitary.

The Primary Plexus of the Eminentia and the Portal Vessels

It has been pointed out several times in this book, that the eminentia mediana in birds is covered by a very dense plexus of capillaries, which are restricted to the surface or have sunk down into furrows. The density of the capillary net is strikingly demonstrated in ink-injected preparations, in which the capillaries occupy up to 50 % of the surface, so the organ is practically covered by a continuous flow of blood (fig. 148). The eminentia plexus extends also to the

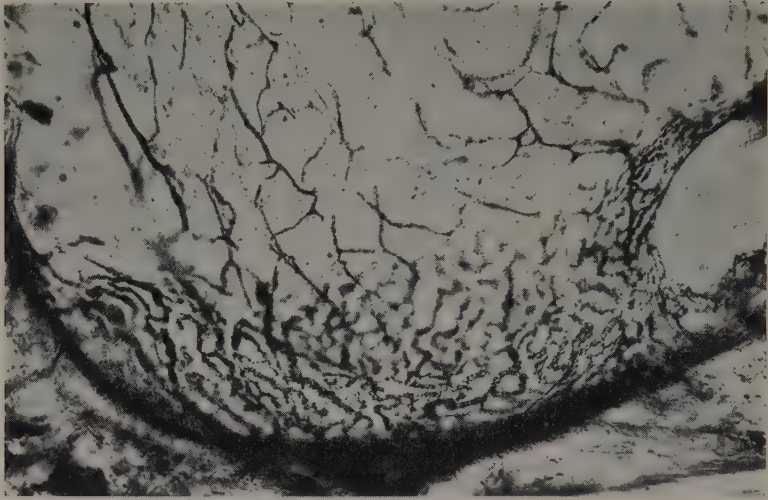


Fig. 148. *Columba livia*, lateral view of the primary plexus of the eminentia mediana showing the density of the capillaries. The wide-spaced net in the upper part of the figure is the internal hypothalamic system. — Ink injection, formalin, thick median section cleared in benzyl benzoate. 80 $\times$.

surface of the infundibular stem, but is usually not so dense there. It is largely independent of the capillary bed of the neural lobe. Only a few vessels can be seen to connect the two systems in the ink-injected specimens. It can also be distinctly seen, in ink-injected specimens as well as in common celloidin or paraffin preparations, that the primary eminentia plexus is almost absolutely isolated from the hypothalamic vascular bed, which extends into the peripheral parts of the eminentia. The hypothalamic capillaries are situated in the deeper parts of the wall, near the ependyma. In the transitional zones of the eminentia such hypothalamic capillaries are fairly common in the nucleus tuberis near the ventricle. The ink-injected specimens show, however, that connections with the superficial primary plexus are extremely few, and the two systems are separated by a rather broad, non-vascularized area (fig. 150, 151). At the periphery of the primary plexus some of its capillaries may communicate with hypothalamic vessels, but as a rule they are connected only with the branches of the infundibular arteries here.

The blood supply to this primary plexus comes exclusively from the so-called infundibular arteries. These vessels are fairly small and variable, but always start from the carotis cerebialis somewhere between the diaphragma sellae and its point of division into anterior and posterior rami. The number of such arteries on each side is 1—3 in the birds examined. In the pigeon (*Columba*) there is one such artery on each side just at the branching-point of the carotis, but it immediately gives rise to several smaller arteries so it is sometimes difficult to state whether there is one or more infundibular arteries (fig. 149). The infundibular arteries supply the surface of the hypothalamus and often send a distinct branch rostrally

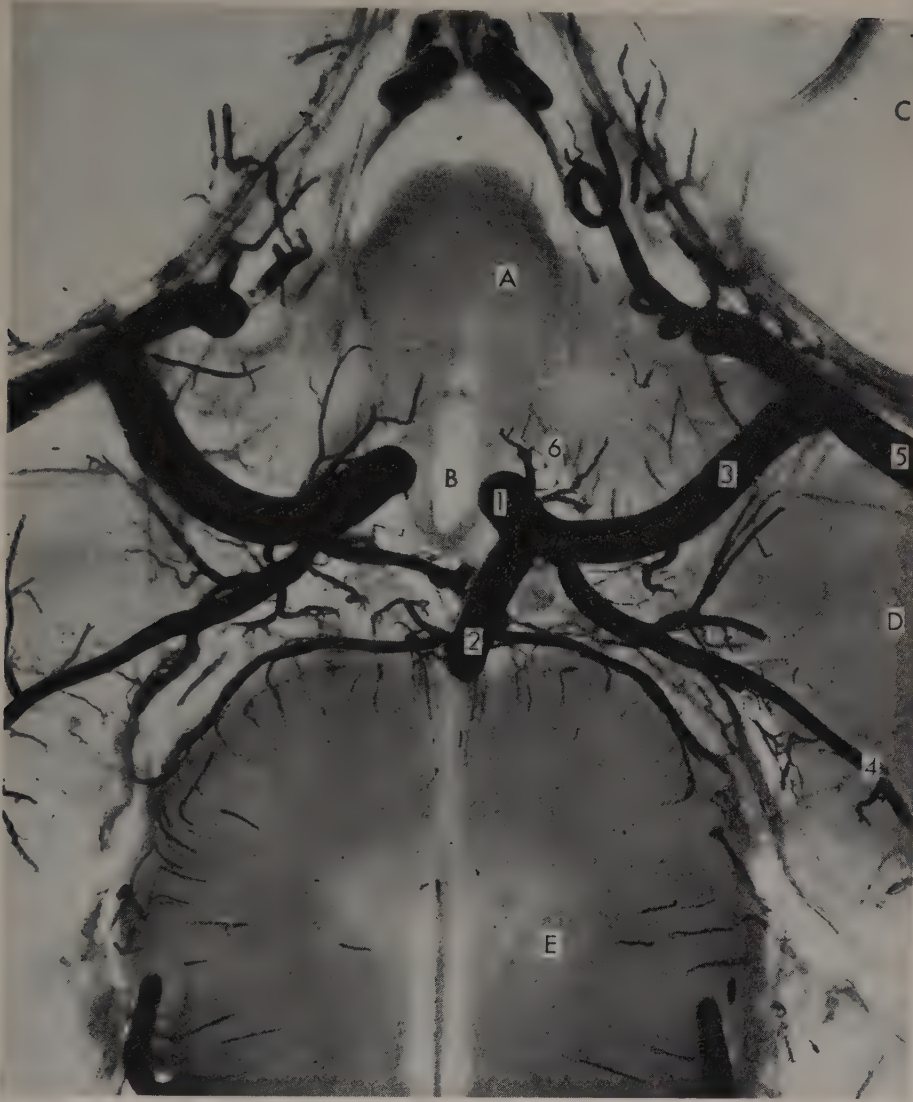


Fig. 149. *Columba livia*, photograph of a horizontal section through the head showing the main arteries. Ventral view.

1 = a. carotis interna, at the point where it penetrates the diaphragma sellae, 2 = ramus posterior of the left carotid, giving rise to the a. basilaris, 3 = ramus anterior of the carotid, 4 = artery to the tectum, 5 = a. ophthalmica externa, 6 = a. infundibularis (partly hidden by the carotis).

A = chiasma opticum, B = ventricle of hypothalamus, C = orbit, D = tectum opticum, E = medulla oblongata.

Fixation in formalin, preceded by starch-cinnobar injection, decalcification, celloidin 2 mm, cleared in benzyl benzoate. 12 X.

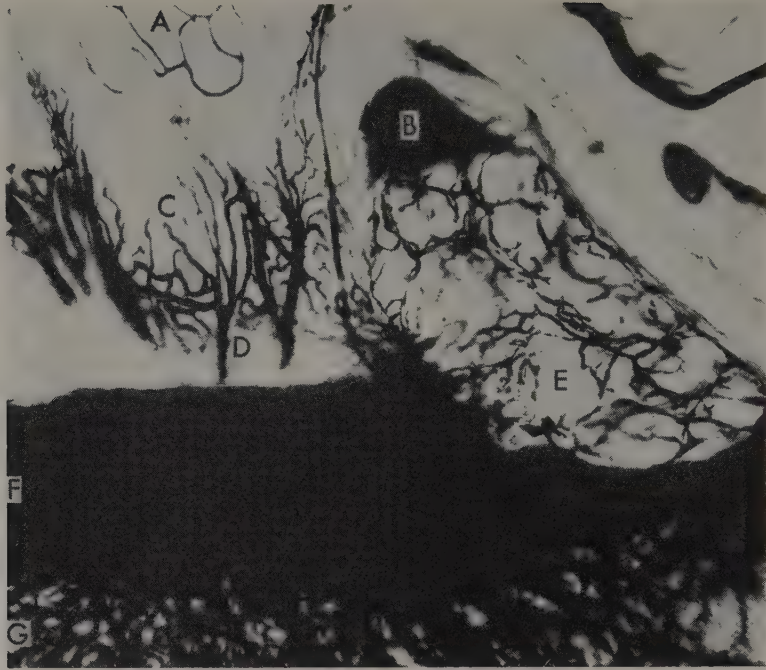


Fig. 150. *Lymnocyrtus minimus*. Paramedian section showing the appearance of the vascular bed in the neural lobe and the origin of the portal vessels in the primary capillary plexus. Some capillaries in the neural lobe can be seen to drain directly into the sinus cavernosus.

A = internal hypothalamic capillaries, B = part of the sinus cavernosus, C = eminentia mediana with the primary plexus, D = portal vessels, E = neural lobe, F = sinus cavernosus, G = sinusoids in the pars distalis. — Ink injection, formalin, celloidin, 160μ , mounted in balsam. $80\times$.

to the surface of the chiasma, but some branches run ventro-medially and end in the primary plexus. This appearance and situation of the infundibular arteries is constant in most investigated birds. In some Passeres, especially *Sylvia*, *Parus*, *Regulus*, *Emberiza*, *Pica*, and *Passer* the infundibular arteries issue nearer the pituitary, just when the carotis penetrates the diaphragma, and a similar situation of the arteries was observed also in *Picus*. Then there are often additional small arteries nearer the brain from the carotis which supply the upper parts of the hypothalamus. In specimens of *Larus arg.* and *Pica* the infundibular arteries are branches of two exceptional arteries which start from the carotis near the rami posteriores and run to the furrow behind the pituitary between the medulla and the hypothalamus (fig. 143).

The capillaries of the primary plexus are largely isolated from the veins of the hypothalamic surface. If connections exist with the hypothalamic vascular bed along the periphery of the eminentia they are few and little important. It can be distinctly observed, however, how the capillaries fuse in the central zone of the

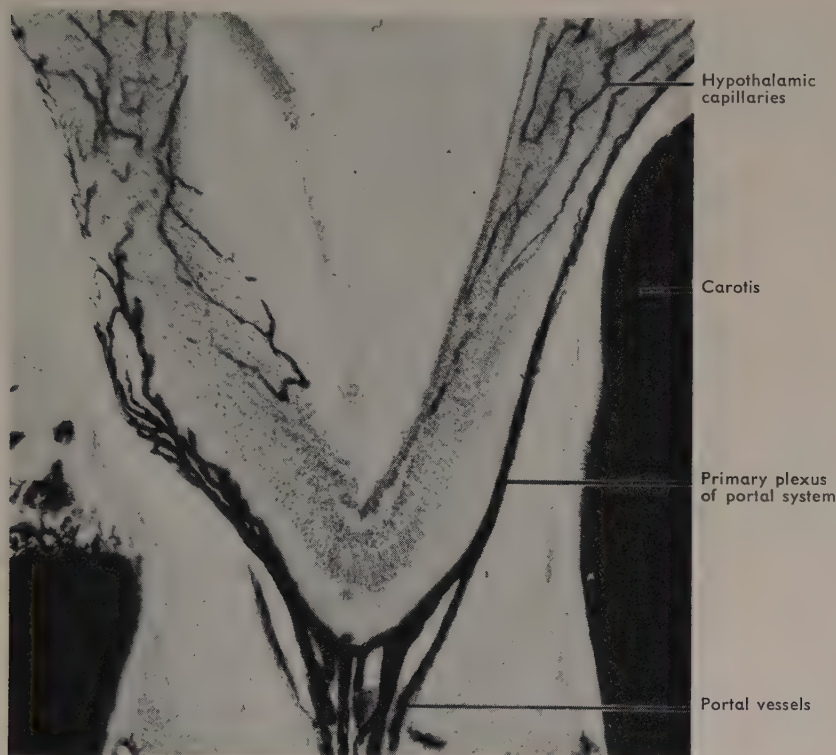


Fig. 151. Transversal section through the eminentia mediana of a pigeon (*Columba*) injected with ink. Note the restriction of the capillaries of the portal system to the surface. The hypothalamic capillaries which are situated deeper in the wall, are not continuous with the superficial system (most distinct to the right, the surface to the left is partly cut tangentially). — Ink injection, formalin, celloidin, 100 μ , Mallory's phosphotungstic acid haematoxylin. 80 $\times$.

eminentia and form larger, thin-walled vessels which run along the surface of the primary plexus and pass towards the portal zone of the pars tuberalis. These draining vessels are the portal vessels, and they can easily be followed all the way along the portal zone to the pars distalis (fig. 150 and 151).

The *portal vessels* are usually relatively few (about 15—30) and have a straight course from the centre of the eminentia to the pars distalis (fig. 27, 30, 151). They are embedded in the epithelium of the portal zone, but some portal vessels usually run to the pars distalis without direct relation to the portal zone. Especially in young specimens it can easily be seen that the epithelial part of the portal zone is paired and forms a plate of cells on each side, whereas the portal vessels form a bundle in the centre.

The morphology of the hypophysio-portal system is thus particularly clear and schematic in birds. The drainage of the primary plexus by the portal vessels is apparent since other veins are practically absent from the eminentia, the portal vessels themselves are long and straight in many species, and their number is

restricted. It is also easy to observe in ink-injected specimens and also in azan and celloidin preparations that the portal vessels branch at both ends: into the primary plexus of the eminentia and into the secondary plexus in the pars distalis resp. The portal vessels and their relation to the primary plexus and to the pars distalis is remarkably constant in all birds, and no variations of importance have been observed.

The Blood Supply of the Pars distalis

In all the investigated birds the portal vessels are the only afferent vessels to the pars distalis, and other affluents have been searched for in vain. The portal vessels enter the pars distalis in the transitional zone between the cephalic and caudal lobes and continue into the gland as straight or arch-shaped enlarged sinusoids which spread to all parts of the gland (fig. 147). There are no capillaries of the common appearance in the pars distalis but only the wide, irregular sinusoids which occupy the spaces between the strings and lamellae of epithelial cells. As described on p. 38 the contact between the blood and the glandular cells is very intimate, the only separating structures are a thin endothelium and a double membrane of an argyrophilic reticulum. The blood escapes from the pars distalis by openings in the periglandular capsule which lead directly out into the surrounding sinus cavernosus. These openings are little complicated. A number of sinusoids, often two or three only, converge near the capsule and fuse into a short common stem which penetrates the capsule and opens on the surface of the organ. In the pigeon such openings are distributed all over the surface, but are most common in the posterior part of the gland, and this seems to be the general rule also among other birds. At such places where the capsule of the pars distalis adheres to the periost of the sella or to the diaphragma sellae the sinusoids do not open directly into the sinus cavernosus but into flattened veins which sooner or later connect with the sinus.

Arteries have not been found in the pars distalis in spite of careful search in ink-injected as well as in celloidin and paraffin preparations. The arteriae ophthalmicae are usually closely attached to the surface of the pars distalis on their way rostrally, but they have been followed carefully in many specimens and no branches to the gland were found. The small arteries from the intercarotic anastomosis also give off some branches to the sella in some specimens. They have never been seen to penetrate the hypophyseal capsule, however, though some small branches sometimes run along the surface of it. These branches are of capillary size, however, and if they sometimes give rise to some minute branches which enter the gland, this kind of supply is presumably practically without importance. Besides, such branches to the sella from the inferior hypophyseal arteries are lacking in many specimens. That the pars distalis lacks arterial supply in birds is shown also by the fact that no arterioles with anything like a media have been seen in all the section series through the pars distalis.

Connections with the capillary bed of the neural lobe are also absent in most specimens. In one injected pigeon I succeeded in finding one single capillary from the neural lobe to the pars distalis, and a similar vessels was found in one specimen of *Larus argentatus*. The absence of arteries in the pars distalis could be distinctly seen also in specimens injected with the starch-cinnober mass which stops in the capillaries. No injection mass was ever seen in the pars distalis, whereas the inferior hypophyseal arteries and their branches in the neural lobe were filled. This observation, which must be regarded as highly conclusive, was made on specimens of *Corvus*, *Pica*, and *Columba*.

Since no other vessels enter the pars distalis it must be concluded that the blood arrives from the portal vessels. This means that the pars distalis of birds does not receive any direct arterial blood. This statement may seem puzzling, but it should be remembered that the portal blood has only passed the dense capillary plexus of the eminentia and is therefore still to be regarded as arterial blood when it reaches the pars distalis. The metabolism in the thin eminentia cannot possibly be of such an extent as to alter the tension of oxygen and carbon dioxide in the large quantities of blood which pass over it.

The Blood Supply of the Neural Lobe

The blood supply of the neural lobe shows considerable variation in birds. Two different sources of the arteries to the lobe have been observed, viz. the aa. infundibulares and the a. hypophyseal inferior. In addition, a quite unique and different arrangement of the arterial supply was found in *Priocella*. The different types will be described separately.

Branches to the neural lobe from the aa. infundibulares were observed mainly in passerine birds (*Regulus*, *Parus*, *Emberiza*, *Riparia*, *Phylloscopus*) but also in *Gallus* (3 specimens), *Lyrurus*, *Diomedea*, *Pelecanoides* and *Oceanites*. Usually there are two symmetrical arteries, one on each side, which branch off from the aa. infundibulares near their origin. The branches destined for the neural lobe are not attached to the eminentia as are the branches to the primary plexus of the portal system, but they run independently in the subdural space along the infundibular stem to the region of the dorsum sellae. In many birds there is a fold of the dura which projects into the sella between the stem and the dorsum sellae, and the arteries therefore run within the subdural space all the way along the posterior surface of the stem to the lobus nervosus (fig. 152). This fold of the dura is particularly distinct in *Gallus*, but in most passerine birds the neural lobe is in immediate contact with the smooth diaphragma sellae, and the arteries thus enter the neural lobe directly after they have penetrated this membrane.

Inferior hypophyseal arteries are present in *Pica*, *Corvus*, *Columba*, *Apus*, *Melanitta*, *Anser*, *Anas*, *Branta*, *Milvago*, *Larus*, *Numenius*, *Tringa*, *Cuculus*, *Dendrocopos*, *Picus*, *Procellaria*, and *Spheniscus*. There may be one or two such arteries, but they invariably arise from the region of the carotic anastomosis in

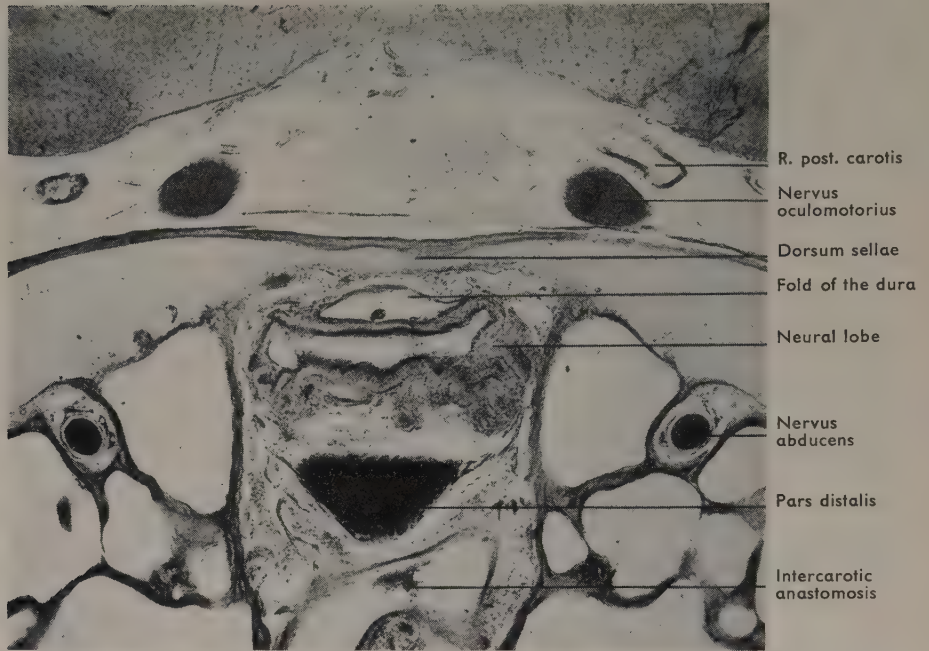


Fig. 152. *Gallus gallus*, newly hatched young. Transversal section through sphenoid region. Note the fold of the dura between the dorsum sellae and the neural lobe. It contains the neural lobe artery (the black dot in the centre). — Bouin, celloidin 60 μ , phosphotungstic acid haematoxylin. 29 $\times$.

the posterior corner of the sella. The simplest structure is present in *Columba*, *Milvago*, *Larus* and the *Anseriformes*. In these species the intercarotid anastomosis emits one or two small arteries which immediately send branches in different directions. A general rule is that one distinct branch runs into the sphenoid straight towards the pharyngeal epithelium. Its direction is towards the anthrum tubarum although it rarely reaches so far, and if remnants of the epithelial stalk are present the artery runs close to the epithelial structures. This was especially distinct in preparations of *Larus* and *Pica* in which the epithelial stalk was fairly well preserved. The branch through the sphenoid in the adult is interesting, since it is present as a much better developed vessel in the embryos and probably is the original vessel from which the inferior hypophyseal artery has arisen (p. 287).

Other branches of the vessels from the anastomosis may enter the sella and supply its periost and connective tissue membranes, and a distinct branch into the dorsum sellae was seen in specimens of *Larus argentatus* and *Columba*. No branches to the epithelium of the pars distalis could be found, however, in spite of careful search.

The inferior hypophyseal artery (or arteries) runs rostro-dorsally along the surface of the dorsum sellae from the anastomosis to the top of the neural lobe.

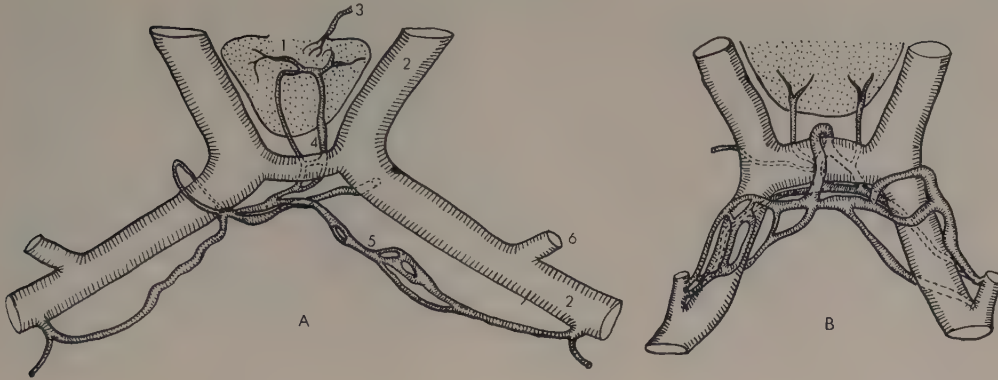


Fig. 153. Drawings showing the complicated origin of the inferior hypophyseal arteries in Corvids. A in *Corvus corone cornix*, ♀ ad., B in *Pica pica*, ♂ juv. Ventral view.

1 = lobus nervosus, 2 = carotis interna, 3 = branch of the infundibular arteries, 4 = inferior hypophyseal arteries, 5 = arterial plexus in the wall of the vena carotis, 6 = a. sphenomaxillaris.

Both specimens had been injected with starch-cinnober, and the drawings were made after the specimens had been cleared in benzyl benzoate and the region of the anastomosis had been exposed. Fig. A about 10×, fig. B about 16×.

Here it is split up into several smaller arteries which enter the lobe and which still, inside the lobe, retain their media for some distance (fig. 147).

In *Pica* and *Corvus* the origin of the inferior hypophyseal artery is more complicated. In these species the vena carotis is surrounded by an arterial plexus which extends from the region of the arteria sphenomaxillaris to the intercarotic anastomosis. The plexus communicates with the carotis by several vessels (fig. 153). The inferior hypophyseal arteries have their origin in this plexus, and they run dorsally of the intercarotic anastomosis to the neural lobe. In the few specimens examined there were two such arteries which appeared to be symmetrical, but this may be accidental. Also a small branch from the infundibular artery to the neural lobe could be seen in one specimen of *Corvus*.

The neural lobe in the specimen of *Priocella* receives its blood supply from two arteries, emitted by the carotis on each side just as it touches the lateral extremities of the lobe. The origin of these arteries is thus within the sella, immediately before the carotis penetrates the diaphragma. This may be regarded as a very rare exception, however, for it has been stated in a large number of specimens that no arteries except the internal ophthalmic ones are emitted by the carotis between the anastomosis and the diaphragma sellae.

The capillary bed of the neural lobe is fairly simple. The vessels are not so wide here as in the pars distalis (fig. 150). In the case of a primitive thin-walled neural lobe they form a net on the surface and in the furrows between the folds of the organ. In neural lobes of the more compact type (e. g. goose, pigeon) they

form a fairly uniform and not very densely spaced lattice throughout the organ. They are drained directly into the sinus cavernosus as are the sinusoids of the pars distalis.

Some Remarks on the Ontogeny of the Pituitary Vessels

My study of the ontogeny of the pituitary circulation is somewhat incomplete since it is based almost exclusively on section series. Injection methods, which are the most adequate procedure in such a case, were used only for some older embryos of *Larus ridibundus* (not listed in table 2). The report is therefore to be regarded as preliminary, and has been included here since nothing is known about these matters before.

Sabin (1917) and Hughes (1934) state that the cerebral supply in embryos of *Gallus* arises from a cloud of capillaries given off from the first (mandibular) aortic arch. Later an anterior and a posterior main vessel are differentiated out from this cloud and form the ramus anterior and posterior of the carotis cerebialis. The branching-point of the carotis cerebialis is thus fixed at an early stage and is distinct in embryos with 29—30 somites. Also the a. ophthalmica interna arises approximately at this stage, and the intercarotic anastomosis is formed in embryos about 4 days old (own observations). The most important fixed points along the carotis in the hypophyseal region are thus recognizable very early, and the arterial system of embryos can be compared with the adult arrangement.

The pituitary circulation begins to become established in 3—5-day embryos of *Gallus* (fig. 154, 156). The hypothalamic part of the peri-neural capillary net is supplied by one or a few dilated capillaries in the 3 and 3.5-day embryos, emitted from the carotis between the a. ophthalmica and the branching-point. These hypothalamic arteries persist to the adult stage and form the aa. infundibulares described above. They are undoubtedly differentiated from the original cloud of capillaries from the first aortic arch. Caudally of the origin of a. ophthalmica interna in the 3.5 day embryo the carotis emits some additional smaller vessels in a rostral direction to the region below the chiasma (fig. 154 A). Later when the intercarotic anastomosis has been formed these vessels attach to the anastomosis or to the carotids just caudally of it. When the chiasma and the oral ectoderm become separated by mesenchyma in older embryos the arteries from the anastomosis retain their contact with the oral ectoderm and thus get a more ventral direction through the prospective fenestra hypophyseos. These vessels remain small in *Gallus* and have even been searched for in vain in some embryos older than 5 days. They are not connected with the hypophysis in this species, but supply the oral roof in younger embryos, and later, when the fenestra hypophyseos has been closed, the sphenoid and the posterior parts of the sella. The inconstant and variable appearance of these vessels from the anastomosis in *Gallus* may be explained by the fact that they do not give rise to any inferior hypophyseal arteries.

In the 3-day embryos in my collection the whole of Rathke's pouch is still in

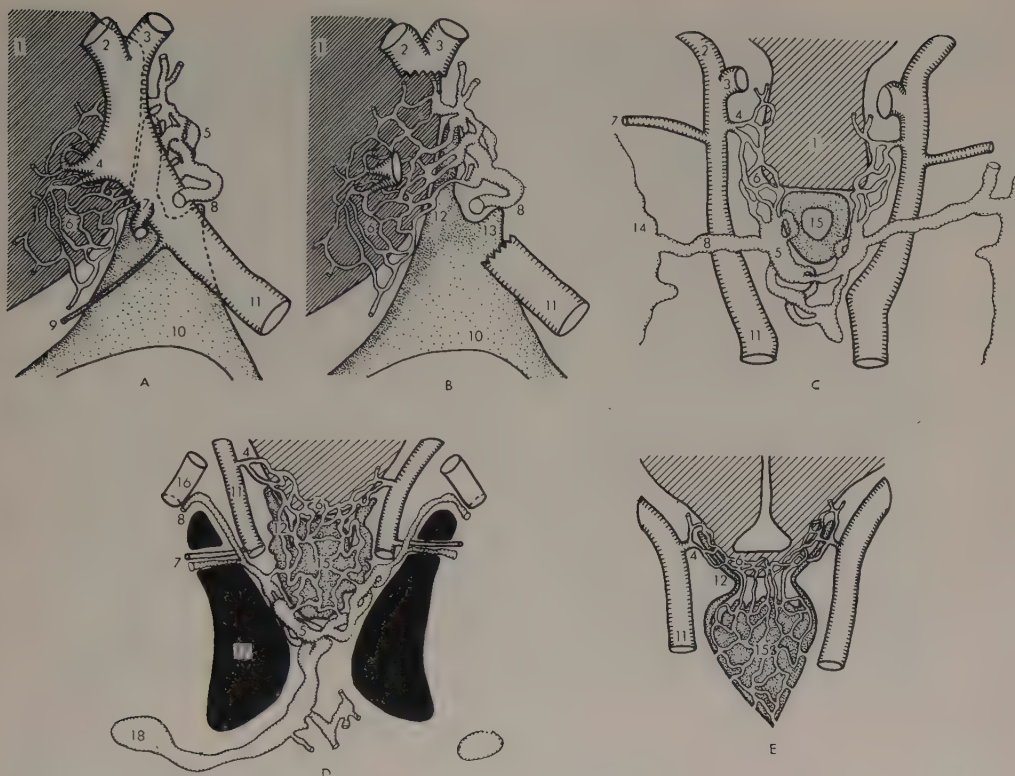


Fig. 154. Diagrams showing the development of the pituitary vessels in *Gallus*.

- A. Embryo, 4 d., hypophyseal region from the left.
- B. The same preparation, a part of the carotid is removed to show the vessels around Rathke's pouch.
- C. Embryo, 3.5 d. Dorsal view of the pituitary region (compare A and B).
- D. Embryo, 9 d., caudal view of the gland and its surroundings. Note the large vein from the sella through the fenestra hypophyseos. Compare fig. 155.
- E. Embryo, 11 days. Caudal view. The cephalic (oral) lobe has withdrawn from the eminentia, and the portal vessels are distinct.

Explanations to all figures: 1 = diencephalon, 2 = ramus anterior and 3 = ramus posterior of the carotis, 4 = a. infundibularis, 5 = plexiform veins behind Rathke's pouch, 6 = capillary plexus on the brain, 7 = a. (and v.) ophthalmica interna, 8 = vena nervi III, 9 = artery from the anastomosis, 10 = oral epithelium, 11 = a. carotis, 12 = lateral lobes of Rathke's pouch. 13 = Seessel's pouch, 14 = v. cerebialis anterior, 15 = central parts of Rathke's pouch. 16 = nervus oculomotorius, 17 = cartilaginous walls of the sella, 18 = head vein.

All figures were drawn on the basis of reconstructions in the same plane as the sections, whereby a series of sections were projected over each other. The plexiform capillaries and veins were not reconstructed in every detail.

intimate contact with the brain. In the 3.5-day embryo, however, the oral lobe of the pouch is separated from the brain and the capillary plexus of the hypothalamus has begun to invade the interspace. Some capillaries continue around the unproliferated Rathke's pouch and fuse with wide, plexiform vessels situated just

behind the top of the pouch (fig. 154 B, C). This post-hypophyseal plexus can be distinctly seen to drain laterally into the anterior cerebral vein (Hughes 1934), near the fusion between this vein and the anterior part of the head vein (Hughes 1934, = primäre Stammvene, v. capitis medialis, van Gelderen 1924). The draining vein is situated dorsally and caudally of the a. ophthalmica interna and becomes closely related to the nervus oculomotorius (fig. 154 D). This vein remains constant to the adult stage and corresponds to the v. nervi III in the adult. The v. ophthalmica interna which accompanies the a. ophthalmica interna to the eye, is definitely absent in the younger embryos. It appears for the first time in embryos incubated for 9 days and can be refound in older embryos up to the hatching-stage, but it remains small (fig. 154 D).

By the 7th day of incubation the v. nervi III is very small and becomes almost vestigial in older embryos, and the v. ophthalmica interna can not drain the sella since it is hardly more than a capillary. The paths to the orbit therefore can not be important for the drainage of the sella from about the 7th day on. In the 7-day embryo and in all older embryos up to the 14th day of incubation there is another venous connection from the sella which undoubtedly has substituted functionally the veins to the orbit. The vascular plexus around the hypophysis is continuous oralwards with wide venous channels which anastomose with the plexus near the oral roof around and rostrally of the base of the epithelial stalk in the 7-day embryo. In the older embryos a mighty venous channel has been differentiated out here, reaching from the perihypophyseal plexus orally through the fenestra hypophyseos in the chondrocranium and, bending laterally near the oral epithelium, fusing with the head vein (fig. 154 D, 155). The large venous spaces here are also, in the older embryos, continuous with the blood caverns in the ossifying sphenoid.

Some small capillaries from the plexus in the sella can be seen to spread caudally along the a. carotis interna in the 8- and 9-day embryos and a fairly rich plexus of smaller vessels can be seen along the carotis up to the 11th day. In the 14- and 15-day embryos these capillaries have become wide and form a mighty venous path from the plexus of the sella caudally to the veins of the occipital region. This is the vena carotis, which evidently begins to function as the chief outlet of the sella in embryos of about 14 days and maintains this function to the adult stage. The veins through the fenestra hypophyseos, which are the main outlet in somewhat younger embryos, disappear when the fenestra is closed from the 14th day on approximately. No such oral veins could be discovered in an embryo aged 15 days, but some smaller remnants of this connection could be found even in an embryo at the hatching-stage.

The sinus cavernosus develops continuously from the small plexiform veins which can be seen behind the Rathke's pouch in the 3.5-day embryo. Up to the 5th day these veins are largely restricted to the region behind the top of the pouch, but in embryos incubated for a longer time they surround the entire hypophysis as a plexus of vessels. They grow in size and number, and from about the 10th day on they begin to attain the appearance of the large venous caverns typical of



Fig. 155. *Gallus gallus*, embryo 9 d. Transversal section through the sphenoid region showing the oral drainage of the sella. — Alcohol-formalin-acetic acid, paraffin 12μ , impregnation according to Palmgren, afterwards stained with eosin. $34\times$.

the adult sinus cavernosus. In the 14-day embryo the sinus cavernosus is so large that the pituitary can be said to be surrounded by a continuous blood sheath as in the adult.

The development of the vessels of the pituitary gland itself is a fairly simple process from a morphological point of view. It was said above that the capillary net of the brain surface had invaded the space between the oral lobe and the prospective eminentia in the 3.5-day embryo, and that vessels from this plexus embrace Rathke's pouch from both sides and fuse with the venous plexus behind the pouch (fig. 154 B, C, 156). It is interesting to note that the course of the blood stream already at this early stage must be from the eminentia, which is supplied by the early formed infundibular arteries, and along the sides of the adeno-hypophysis to the veins. When Rathke's pouch begins to proliferate in 5- and 6-day embryos the capillaries encircling it become more numerous (split up?) and are included in the proliferating epithelium, and this process goes on until the rich plexus in the adeno-hypophysis is formed (fig. 154 D). All the time it can be seen that the vessels in the developing pars distalis are continuous with the net of the surface of the eminentia on the one side and with the venous plexus behind the adeno-hypophysis on the other side.

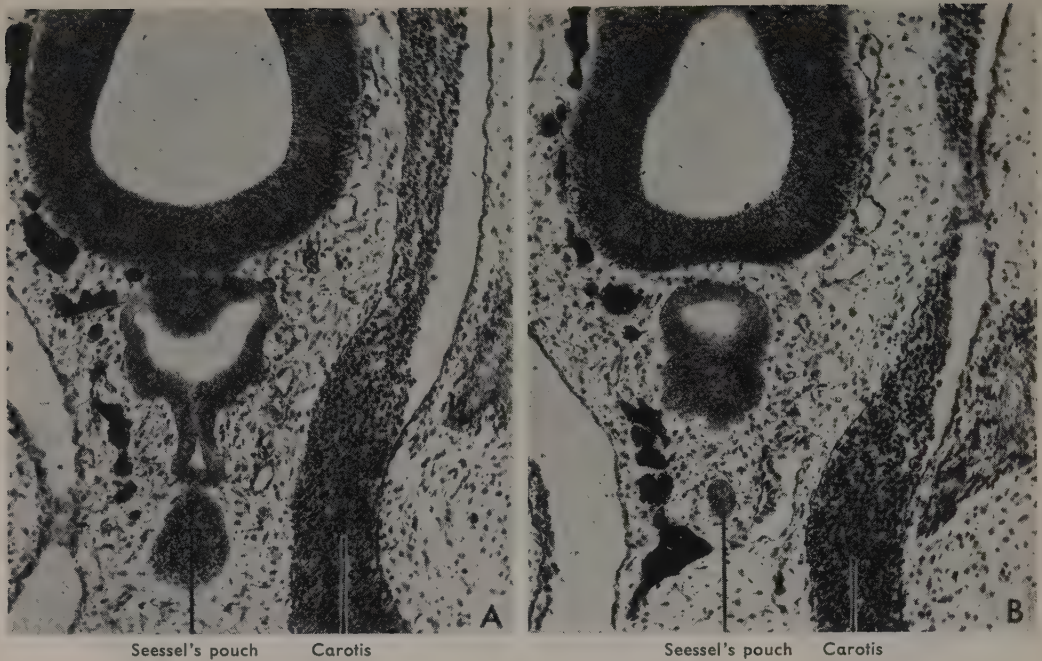


Fig. 156. *Gallus gallus*, embryo 3.5 d. Horizontal sections through the region of Rathke's pouch parallel with the plane of the carotids (cf. fig. 154: A—C). The vessels on the left side have been marked with ink on the photograph. In A the capillary plexus of the brain can be seen to continue along the sides of Rathke's pouch. In B, which is 30μ dorsally of A, it can be seen that these capillaries drain into the plexiform veins behind Rathke's pouch. — Bouin, paraffin 6μ , azan. $105\times$.

It was suggested by Harris (1947) that the pars tuberalis serves to establish the vascular contact between the eminentia and the pars distalis. Though this seems to be a reasonable view it is very difficult to find any support for it in the embryonic development. The vessels which first encircle Rathke's pouch have no specific relation to the lateral lobes. The oral lobe is attached to the prospective eminentia in the first half of the incubation period and there are no discrete portal vessels, but the plexus of capillaries on the eminentia is directly continuous with the one of the adenohipophysis. The lateral lobes project laterally of this vascular system to the brain surface, and scattered capillaries run along their medial surfaces, but the majority runs from the eminentia to the oral lobe in a more median position independently of the lateral lobes.

In embryos incubated for 9 days and more the oral lobe begins to withdraw from the eminentia, and the uniting capillaries are then stretched out and form the straight and typical bundle of portal vessels (fig. 154 E). The lateral lobes retain the contact with the brain and are therefore also stretched out and invest the vascular bundle from both sides, yet without direct contact with the portal vessels. This relation between portal vessels and pars tuberalis can be seen also in the

newly hatched chick, but later the portal zones of the pars tuberalis approach the mid-line and enclose the portal vessels between them. Also in adult birds it can often be distinctly seen, however, that the zona tuberalis consists of two cell strings which invest the string of portal vessels from the sides.

The vascular system of the adenohypophysis is thus practically definite in embryos from the 9th day on. That of the neural lobe is formed directly from the general capillary plexus of the brain wall, and some capillaries are enlarged so as to form the arteries which carry the arterial blood directly to the neural lobe from the infundibular arteries. Distinct arteries of this kind were observed in the 15-day embryo and in older stages.

The development of the vessels in *Larus ridibundus* does not differ very much from that in *Gallus*. The arteria ophthalmica interna is present already in the 7.5 mm embryo and also the infundibular arteries and the rostrally directed vessels which develop into the arteries of the intercarotic anastomosis. The latter is established in an embryo of 13.6 mm (CR) and in all larger ones except in a 14 mm embryo. The only distinct difference from *Gallus* in the development of the arterial system is that the arteries from the intercarotic anastomosis become connected with the neural lobe. Just as in *Gallus* these arteries are originally directed rostrally towards the oral roof beneath the chiasma, and in older embryos their main branches descend through the fenestra hypophyseos to get into this area. In embryos as small as 18 mm (CR) these arteries can be seen to emit one or more branches dorsally to the neural lobe and these inferior hypophyseal arteries can be seen in most larger embryos. When the fenestra hypophyseos is closed in older embryos these arteries from the anastomosis can sometimes be followed through the sphenoid to the oral roof, but in some specimens the branches do not reach outside the sella. The branches to the neural lobe are, however, distinct and large in all embryos about the hatching-stage. Thus it seems probable, on account of the embryonic development, that the inferior hypophyseal arteries have arisen secondarily as branches from arteries originally supplying the oral roof.

The veins develop exactly in the same way as in *Gallus*. The venae nervi III are the only outlet of the sella from the 7.5 mm stage to the 18 mm stage approximately. Large veins through the fenestra hypophyseos in the chondrocranium connect the sella with the head veins in embryos from about 18 mm CR to about 28 mm CR (TL 45). The vena carotis begins to develop as a delicate capillary plexus along the carotis in 18—20 mm embryos and forms a large venous pathway in all embryos larger than 25—28 mm (CR). The oral outlet of the sella through the fenestra hypophyseos is reduced very much or is absent in all embryos larger than 29 mm (CR). The v. ophthalmica interna is small but can be seen in embryos measuring 18 mm (CR) or more.

The vascular system of the pituitary proper begins to develop in the 7.5 mm embryo. The capillaries of the hypothalamus have not reached the median line between the prospective eminentia and the Rathke's pouch, but some of its capillaries invest the pouch from the side and are emptied into a venous plexus behind

the top of the pouch, from which the venae nervi III start. The vessels encircling the pouch become split up and multiplied and form the capillary bed of the adenohipophysis, when the latter begins to proliferate. The capillary net of the eminentia and that of the adenohipophysis are completely continuous till about the 25 mm stage (CR), at which time the oral lobe begins to withdraw from the eminentia and the portal vessels are stretched out. It was not possible to find any certain relation between the development of this vascular pattern and the pars tuberalis (lateral lobes).

In *Riparia riparia* the initial development of the pituitary vascularization is the same as in the preceding species, but the further development is slightly different (cf. fig. 157). No hypophyseal vessels could be discerned in embryos smaller than 6 mm CR. In the embryo of 6 mm there is a hypothalamic plexus which sends capillaries around Rathke's pouch to the veins behind the top of the sella. These are continuous with the vv. nervi III, which have the same course and are emptied in the vv. cerebrales anteriores on each side near the connection with the anterior head vein. This first differentiation is identical in the three species. There are also arteriae ophthalmicae from about the 6- or 7-mm stage, and arteries which originate caudally of this point and which form the branches from the anastomosis. The latter is established in embryos measuring 7—9.5 mm CR and is present in all older specimens.

The development of the aa. infundibulares and the arteries from the anastomosis is not different from that in *Gallus*. No inferior hypophyseal artery is formed, but the neural lobe is supplied from the a. infundibularis.

The vena nervi III is the outlet of the hypophyseal region up to the 7—10.5 mm stage (CR) but later it becomes vestigial. It seems to be directly succeeded by the v. carotis, which begins to develop as a capillary plexus in 9—10 mm embryos and consists of very wide veins in some 10.5 mm. embryos. The v. ophthalmica interna is small in the embryos. It may be noted that the oral connections via the fenestra hypophyseos to the head veins are insignificant in all embryos of *Riparia* except in one measuring 14 mm (CR). It is thus probable that the second phase in the development of the venous system in the other two species, characterized by the orally directed veins, has been eliminated in *Riparia*. It must be noted, however, that I searched in vain for outlets from the sella in two continuous section series of embryos measuring 8.5 mm CR. Maybe the vessels have collapsed and therefore could not be recognized, for some kind of circulation in the pituitary must be expected at this stage. Absence of circulation would conflict with the statement that the gland is functional at this stage (p. 123).

The development of the adenohipophyseal circulation and the portal vessels is exactly the same as in the other two species. The oral lobe begins to withdraw from the eminentia in 12—14 mm embryos, and the portal vessels then form a bundle of straight channels from the eminentia to the adenohipophysis. The portal vessels have no close relation to the lateral lobes (pars tuberalis), but they are invested by the portal zone in post-hatching stages.

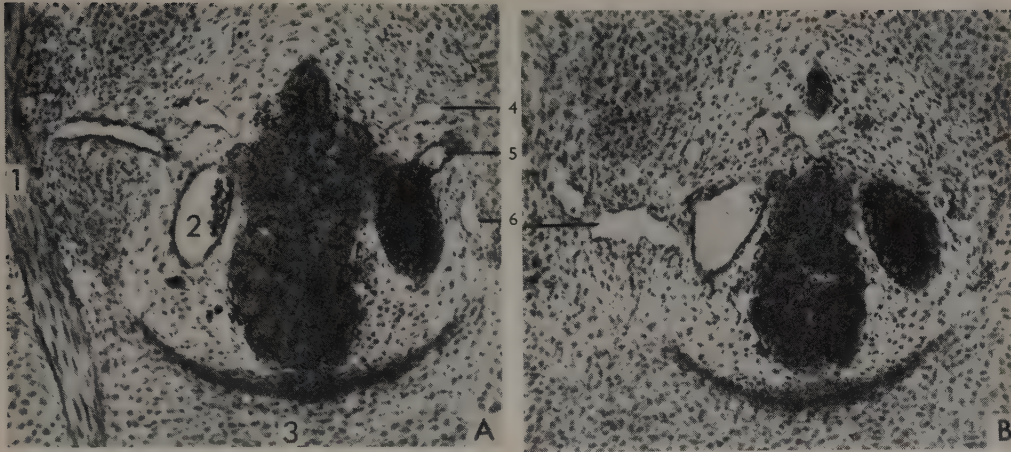


Fig. 157. *Riparia riparia*, embryo, CR 10.5 mm. Horizontal sections through the pituitary region. The rostral parts are upwards in the figure. The vena nervi III and the v. ophthalmica interna are still fairly large in this specimen. Figure A shows a section situated 32μ dorsally of the one in fig. B. Confer fig. 154.

1 = nervus oculomotorius, 2 = a. carotis interna, 3 = blastem of dorsum sellae, 4 = v. ophthalmica interna (distinct to the left in fig. A), 5 = a. ophthalmica interna, 6 = v. nervi III.
— Susa, paraffin 8μ , azan. $115\times$.

The study of the embryonic differentiation of the pituitary vessels thus shows:

1. That the adenohypophyseal plexus, the eminentia plexus and the portal vessels are formed by specialization of a certain part of the capillary plexus of the brain. The originally continuous system of capillaries is differentiated into two, one on the eminentia and one in the pars distalis, when the oral lobe of Rathke's pouch withdraws from the brain. The two plexus formed are connected by some of the original capillaries which are drawn out when the gap between the oral lobe and the eminentia arises. These straight vessels develop into the portal vessels of the adult. No certain relation between lateral lobes and portal vessels could be observed.
2. That the arteriae infundibulares arise very early from the bundle of capillaries from the first aortic arch. The arteriae hypophyseae inferiores are to be regarded as secondary branches from arteries originally destined for the oral roof beneath the chiasma.
3. The venous outlet from the sella shifts twice in *Gallus* and *Larus*. The first outlet is established by the v. nervi III, the second by veins via the fenestra hypophyseos to the head vein, and the third and permanent by the vena carotis. In *Riparia* the second connection via the fenestra hypophyseos seems to have been eliminated. The v. ophthalmica interna arises early but remains small in the three investigated species.

Point 3 is in disagreement with Hughes' statement to the effect that the plexus in the sella turcica lacks connection with the venous system in the younger embryos, but Hughes probably did not pay any particular attention to the pituitary region.

The only investigation of the embryology of the hypophysio-portal system of vertebrates to be found in the literature seems to be that of 'Espinasse (1933). According to this author, the portal system of the human pituitary develops from branches of the internal carotids, which run to the prospective hypothalamus. On the way a stretch of them is included in the pars distalis and is split up to form the sinusoids of this organ. The next part of the arteries between the gland and the brain is not split up so extensively and forms the portal vessels, and the final ramification of the arteries on the surface of the eminentia gives rise to the capillary net here. According to 'Espinasse the direction of the blood streams is from the pars distalis to the eminentia as soon as the portal vessels appear, and the sinusoids of the pars distalis would then be something like an arterial rete mirabile. Since the vessels connecting the pars distalis with the eminentia are derived from arteries, 'Espinasse rejects the term "portal vessels". It is hardly necessary to point out that my results on avian material deviate fundamentally from 'Espinasse's statements. In birds there are no arteries involved in the development of the adeno-hypophyseal circulation, and since the primordial vessels are connected with the veins behind Rathke's pouch at one end and with the capillaries on the brain at the other, there is only one possible direction of the blood stream: from the brain via Rathke's pouch to the large veins. Though the conclusions in this paper are based on avian material they indicate that the development of the hypophysio-portal system in mammals ought to be re-investigated.

Comparison with the Pituitary Vessels in Other Vertebrates

In the vascular system of the amniote pituitary we may recognize a set of very constant features and another set of variable ones. The portal system belongs to the first category. In all amniotes it consists of a dense plexus of capillaries on the eminentia mediana, drained by wide portal vessels which pass to the capillary bed of the pars distalis (Green 1951). This fixed arrangement of the portal system in the pituitary was also found by the present author in snakes (*Vipera*, *Tropidonotus*, *Coronella*) and lizards (*Lacerta*) studied by injection technique. As shown by Green (1947 a) such a portal system is more or less differentiated in amphibians and my own preparations of amphibians (*Rana temporaria*, *Bombina bombina*, *Salamandra maculosa*) fully corroborate his descriptions. Another constant feature is that the vascular bed of the neural lobe is independent of the portal system and is drained directly into the sinus cavernosus or, in the absence of a typical sinus, into the veins of the region. The fishes are difficult to compare (cf. Green 1951).

It is remarkable that this arrangement of the capillary nets and the portal vessels proves to be conservative in amniotes, whereas the larger vessels, arteries as well

as veins, vary extensively. This indicates that the portal system has a fundamental functional significance.

The general course of the internal carotid and its branches cannot be reviewed here, and the reader is referred to Hafferl (1933). The intercarotic anastomosis which is so characteristic of birds, is lacking in all mammals, in snakes and in *Sphenodon*, whereas it is present in crocodiles and chelonians (Shindo 1914, Green 1951). It is lacking also in most lacertilia in my collection, and the only exception was found in three specimens of *Chamaeleon ?gracilis* which have a distinct cross-connection behind the pituitary. Such an anastomosis is not present in amphibians (Green 1947 a, own observations), but this is not surprising since the course of the carotis is very exceptional in these animals.

The origin of the a. ophthalmica interna varies considerably in reptiles and mammals (cf. Hafferl 1933). In birds this artery regularly leaves the carotis within the sella turcica, but in many amniotes it is a branch of the ramus anterior of the carotis. Great variations in the appearance of the circulus arteriosus Willisi have also been described. A general feature is, however, that the carotis divides into the anterior and posterior rami as soon as it reaches the brain, and this point of division may thus be referred to when the origin of the hypophyseal arteries in different animals is compared.

The primary plexus of the hypophysio-portal system, which covers the eminentia, is supplied by arteries of very different origin in different vertebrates. They may leave the carotis just when it penetrates the diaphragma sellae (some birds, monotremes, Hanström and Wingstrand 1951), they may originate near the dividing-point of the carotis (many birds, rat, rabbit, Macacus; cf. Landsmeer 1951, Wislocki 1938 a, Harris 1947), or they may come from the ramus anterior of the carotis far from the point of division (snakes, lizards; own observations, see fig. 158).

The arteries to the neural lobe vary still more. It was shown above that the neural lobe arteries in birds may come either from the intercarotic anastomosis behind the pituitary or from the infundibular arteries above the pituitary. In *Homo*, *Macacus*, *Felis*, and other mammals there are inferior hypophyseal arteries to the neural lobe, leaving the carotis when it is still inside the sinus cavernosus. In other mammals as *Tachyglossus* and *Ornithorhynchus* the arteries to the neural lobe come from the neighbourhood of the point of division of the carotis and in the rabbit from the point where this artery penetrates the diaphragma sellae (cf. Romeis 1940, p. 474, Wislocki 1938 a, Morato 1939, Hanström and Wingstrand 1951, Wislocki and King 1936, Harris 1947). The latter arrangements are similar to that described above in *Gallus* and some passerine birds. In *Mus* the artery to the neural lobe usually comes from the ramus posterior of the carotis (ramus communicans posterior; Landsmeer 1951).

In *Tropidonotus* and *Vipera* (fig. 158) the neural lobe has a double supply. One small artery comes from the infundibular arteries and runs along the stem to the neural lobe, and another artery with the same destination leaves the carotis just as it enters the sella (own observation). In amphibians the arteries to the neural

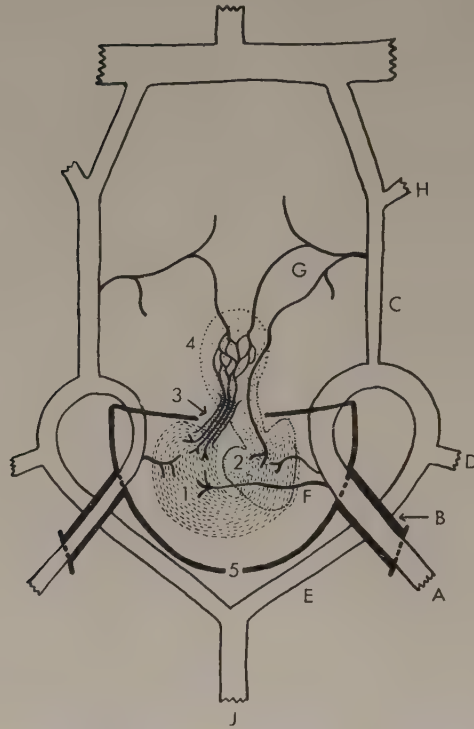


Fig. 158. Diagram of the vascular supply of the pituitary in *Vipera berus*. The vessels drawn in this figure have been seen in numerous section series of large embryos and also in several injected adult specimens. The pituitary is asymmetrical.

A = carotis interna, B = the part of the artery situated inside the canalis caroticus, C = ramus anterior, D = ramus medius, and E = ramus posterior of the carotis, F = inferior hypophyseal arteries, emitted from the carotis just when it enters the sella, G = aa. infundibulares, H = a. cerebri media, J = a. basilaris.

1 = pars distalis, 2 = neural lobe, 3 = "pars terminalis" with portal vessels, 4 = eminentia mediana with the primary plexus of the portal system, 5 = internal wall of the sella turcica.

Confer O'Donoghue (1912) and Grodzinski (1928).

lobe leave the transversal artery behind the pituitary (Green 1947 a, own observations). The situation of this transversal vessel is such that it falls within the region of the ramus posterior of the carotis in higher vertebrates.

The previous parts of this chapter justify the conclusion that no arterial blood arrives directly into the pars distalis of birds, but all of it must pass the portal system before it enters the gland. This is, however, no fixed rule in amniotes. My preparations of *Vipera berus* distinctly show that the pars distalis receives a pair of direct arteries coming from the carotis just when it enters the sella (fig. 158). Direct arteries to the pars distalis have also been described in mammals, but opinions differ as to their existence (cf. Romeis 1940, p. 482, Brooks and Gersh 1940, Morin and Bötner 1941, Harris 1947). Landsmeer (1951) has given rather

convincing evidence against the existence of arteries to the pars distalis in the rat. My own experiments with *Rana temporaria*, referred to below, have convinced me that no arteries enter the pars distalis in this animal.

The venous system is known to be variable and is usually a poor object for detailed comparative research. The veins of the pituitary region show great variations in different amniotes. Thus, for instance, the veins around the pituitary form a sinus cavernosus in birds as described above. In mammals there is also a (non-homologous) cavernous sinus on each side of the pituitary, but it does not seem to surround the pituitary as intimately as in birds (cf. Shindo 1915, Romeis 1940, own observ.). Among the reptiles we find an enlarged transversal vein behind the pituitary in lacertilia, snakes and in *Sphenodon*, but no real sinus cavernosus (own observations, Shindo 1915, Sæve-Söderberg 1946). A cavernous sinus is formed around the intercarotic anastomosis in Chelonia and Crocodilia (Green 1951).

An extensive investigation of the homologies of the sinus cavernosus in mammals and reptiles was made by Shindo (1915). He based his conclusions on the appearance of the embryonic veins, and could state that the primordia of the venous vessels in the pituitary region have the same situation and can be homologized in all investigated species of mammals and reptiles (*Oryctolagus*, *Lacerta*, *Chelone*, *Emys*, *Crocodilus*). In the young embryos there is a distinct transversal "vena retrohypophyseæ" behind Rathke's pouch, and this vein drains laterally into the v. capitis medialis on each side, near the junction of the latter with the v. cerebialis anterior. The further development of these vessels is highly different in mammals and reptiles since the v. capitis medialis is dislocated into the brain-case together with the cavum epiptericum in the former whereas it remains extracranial in the latter (Gaupp 1905, Shindo 1915, v. Gelderen 1925, p. 559). Shindo states that the sinus cavernosus in mammals is formed by this intracranial part of the v. capitis medialis, and that the sinus intercavernosus posterior, which connects the two sinus cavernosi behind the pituitary in the adult, is derived from the v. retrohypophyseæ in the embryo. The reptilian homologon of the sinus cavernosus in mammals would then be that part of the vena capitis medialis which is situated in the lower part of the orbit, above the processus basipterygoideus. Van Gelderen (1924 b, p. 500, and 1925, p. 547) points out, that a continuous development of the v. capitis medialis into the sinus cavernosus can be seen only in the Monotremata and the Primates, whereas it arises secondarily after the vein has disappeared in the other mammals. He agrees, however, with Shindo in the essential questions concerning the homologies (v. Gelderen 1925, p. 559).

As shown above the vena nervi III in bird embryos arises as a transversal vessel behind Rathke's pouch and drains the veins of this region into the v. cerebialis anterior at its junction with the v. capitis medialis. It thus corresponds perfectly to the v. retrohypophyseæ in Shindo's (1915) embryos of reptiles and mammals. Especially Shindo's figure of this vein in a rabbit embryo (Textfigur 5) is almost identical with a section through a bird embryo (my fig. 154 C). It was also described

above how the sinus cavernosus in birds arises from the median parts of this transversal vein and its homologon in mammals must therefore be the median parts of the sinus intercavernosus posterior. Inversely the mammalian sinus cavernosus must be homologous with some orbital part of the v. capitis medialis in birds, that will be the same as the v. ophthalmica of Neugebauer (1845). A comparison with reptiles gives the result that the sinus cavernosus of birds might correspond to the median parts of the vena (retro-)hypophyseal in the reptiles, and when a sinus cavernosus is formed as in chelonians and crocodiles it may be regarded as homologous with the avian one.

The veins connected with the sinus cavernosus in birds should be briefly mentioned. The vena carotis, which is large in birds, arises late during the ontogenesis and gives the impression of a secondary vessel without great morphological significance, and this view is supported by comparisons with other amniotes. A vena carotis from the sella through the canalis caroticus is present in some mammals, e. g. *Acrobatus*, *Didelphis*, *Canis* (Shindo 1915) and *Ornithorhynchus* (Hanström and Wingstrand 1951). In man this vein is represented by a "plexus venosus caroticus internus" without connections with the extracranial system, and in other mammals the drainage of the sinus cavernosus into the extracranial veins is established by other veins (cf. Shindo 1915). In the lizards and snakes in my collection there is no distinct vena carotis and the chelonians and crocodiles seem to be insufficiently known in this respect.

The other two veins in birds, the vena nervi III and the vena ophthalmica interna, also deserve special mention. The former was said above to be homologous with the vena retrohypophyseal in mammalian embryos and consequently corresponds to the lateral parts of the sinus intercavernosus posterior in adult mammals. The homologon of the v. nervi III in reptilian embryos is also the v. retrohypophyseal, and this vein runs from the pituitary on each side through the foramen metopticum to the vena capitis medialis. In adult reptiles this vein is sometimes secondarily connected with other vessels (cf. Grosser and Brezina 1895, Bruner 1907, Shindo 1915, Grodzinski 1928, Säve-Söderberg 1946). The homologies of the v. ophthalmica interna are difficult to discuss because the artery along which it runs is so variable.

The Functional Significance of the Pituitary Vessels

The vascularization of the neural lobe does not raise any difficulties for a functional interpretation. The capillary net is in intimate contact with the neural lobe tissue and can easily transport away the secretions. It receives direct arteries and is drained directly into the sinus cavernosus and is thus independent of the other hypophyseal parts. It seems little probable that secretion into the ventricle of the neural lobe can be of a functional importance. Though this way is open in birds with a wide recessus infundibuli there are no serious objections to the opinion that the dense capillary net on the surface can wash away the secretions in these

species too. In birds with a neural lobe of the compact type the distance from most parts of the organ to the ventricle is so great that only the vascular way of escape of the hormones seems reasonable. This applies also to many mammals and snakes with a compact neural lobe.

The supply of the eminentia mediana and the adenohypophysis is intimately connected with the portal system, and before any conclusions can be drawn as to the function it is necessary to ascertain the direction of the blood stream in this system. Investigators of other animal groups have given very different answers to this question. Especially the school of Popa (cf. Popa and Fielding 1935, Popa 1937) have maintained that the direction of the blood stream is from the adenohypophysis towards the brain in the portal vessels, whereas a number of other authors are of the opinion that it is just the reverse, in agreement with Wislocki and King (1936).

In birds the structural relationships of the hypophysiportal system are particularly clear and leave no doubt that the direction is from the adenohypophysis to the eminentia. The facts supporting this view are summarized below:

1. *No other way of blood supply to the pars distalis than from the portal vessels can be discovered.* The sinusoids of the pars distalis can be distinctly seen to be continuous with the portal vessels. The sinusoids also open directly into the surrounding sinus cavernosus through the surrounding capsule. If other vessels to the pars distalis existed in birds, they would readily be discovered in the sections with the gland in situ since the pars distalis is so isolated from other organs with a rich vascularization. Especially the specimens injected with the starch-cinnober suspension, which fills all arteries and even small arterioles, proves that no arteries enter the pars distalis. The theoretical possibility that blood could enter the gland from the sinus cavernosus need hardly be discussed. *Thus, if the pars distalis receives blood whatever — and this seems reasonable indeed — this blood must come from the portal vessels.* Since the portal vessels are few and their appearance and mode of junction with the eminentia capillaries is the same, it cannot be believed that the direction of the blood should be upwards in some vessels and downwards in others. A transfer of blood from the pars distalis to the eminentia is thus highly improbable — it is tempting to say impossible.

2. The capillary plexus of the eminentia has no or very small connections with the superficial veins of the hypothalamus, but are directly continuous with the portal vessels. Along the periphery the eminentia plexus receives numerous arterioles which are branches of the infundibular arteries. The number of these arterioles shows that a considerable quantity of arterial blood must be transported to the eminentia per unit of time. The small and few capillaries which connect the eminentia plexus with the common hypothalamic capillaries along its periphery cannot possibly represent an effective drainage, since they are insignificant in comparison with the arterial supply. The only vessels which have such a size that they correspond to the supplying arteries are the portal vessels. *It must be concluded therefore that the blood is transported away from the eminentia in the portal*

vessels. The suggestion that blood is transported to the eminentia in the portal vessels will lead to the absurd conclusion that there are two mighty blood-supplying systems to the organ but practically no drainage.

This conclusion holds for birds whereas the structure of the vascular system in such animals as e.g. snakes hardly allow an equally definite statement (fig. 158). More and more evidence for a transport of the blood from the eminentia to the pars distalis in different animals has accumulated in the last few years, however, and the constant morphology of the portal system may justify the opinion that this direction of the portal blood is common to all amniotes and amphibians.

Definite evidence for the direction of the blood stream in the portal vessels of amphibians has been presented by Houssay, Biasotti and Sammartino (1935) and Green (1947 a), who exposed the hypophyseal region and observed the movements of the blood corpuscles under a dissecting microscope. Not knowing the said publications I made the same experiments in 1947 and 1948 with *Rana temporaria* (not published) and could distinctly see the blood corpuscles passing from the eminentia through the portal vessels to the pars distalis. I further coagulated the blood in the portal vessels by means of a thin glass capillary filled with alcohol and then injected the vascular system with ink from the heart. It could then be seen that the pars distalis was uninjected in all specimens in which the portal vessels had been completely blocked. The neural lobe and the pars intermedia were always filled with ink since they receive a separate arterial supply (cf. Green 1947 a).

Evidence for the direction of the blood in reptiles and birds seems to be lacking whereas some experiments have been made with mammals. Green (1948) exposed the pituitary in dead rats and observed the vessels while the animal was injected with ink. Harris (1948 a) made the same experiments with anaesthetized rats. The ink appeared first in the eminentia and successively spread along the portal vessels to the pars distalis. Identical experiments with rats were made by Barrnett and Greep (1951) and the same result was obtained. Further Green and Harris (1949) worked out a method by which they could see directly that the blood corpuscles pass towards the pars distalis in the portal vessels of the living rat.

Though these experiments are most convincing, a number of other observations support the same view. Incomplete ink injections often give the result that some vascular paths are continuously filled from the primary plexus in the eminentia through the portal vein into the pars distalis, and there is always a continuity with the eminentia when ink appears in the pars distalis (Green and Harris 1947). This was seen also in some birds (*Lyrurus tetrrix*) during the present investigation. Further Morato (1939) injected fat into the vessels of the cat and the dog and observed the distribution of the fat emboli in the pituitary vessels. He concludes that the vascular supply of the neural lobe is independent of that of the pars distalis and that the direction of the blood in the portal vessels is downward. Anastomotic vessels between the posterior lobe and the pars distalis were seen. It should be mentioned also that Landsmeer's (1951) detailed study of the vessels

of the rat pituitary showed that the above opinion concerning the direction of the blood in the portal vessels must be right, but also that the vascular beds of the posterior lobe and of the pars distalis anastomose in this species.

We have, thus, good reason to hold that the blood passes from the eminentia mediana to the pars distalis in the portal vessels. The functional implications of this statement have been discussed at length in chapter XII. It is clear, that a transport of secretory products can take place from the eminentia to the pars distalis via the portal vessels but not in the opposite direction. It is then difficult to interpret the results obtained by Borell (1945). He found an exceptional concentration of thyreotropic hormone in the tuber cinereum and in the plexus chorioideus of different mammals. Further, he noted an increase of the thyreotropic activity in these parts after rats had been exposed to cold and the output from the pars distalis consequently had been increased. No such increase could be stated when varying amounts of the hormone were injected into the animals. Borell therefore concludes that the thyreotropic hormone is transported via the stalk of the hypophysis to the tuber cinereum. The said facts concerning the vascular system indicate, however, that there may be another explanation, or that the direct migration of thyreotropic hormone into the brain is restricted to species with a short distance from the pars distalis to the eminentia. Then it may be possible that some small proportion of the hormones diffuses through the tissues to the neurohypophysis, but this can hardly be the normal way of escape of the hormones of the pars distalis. In birds such a diffusion of hormones from the pars distalis to the ventricle of the neurohypophysis is impossible.

CHAPTER XIV

Some Notes on the Surroundings of the Pituitary

Most structures surrounding the pituitary in birds have been mentioned in the preceding chapters of this book, but no general survey of the pituitary region has been made. Since this may be of some value to experimental work I have added a few lines here on the hypophyseal region. No comparisons with other animals have been made, however, for this is beyond the scope of this book.

The material used for this part consists of celloidin and paraffin section series with the pituitary in situ (table 1) and also of skulls of *Cygnus olor*, *Anser fabalis*, *A. anser*, *Anas platyrhynchos*, *A. crecca*, *Larus ridibundus*, *L. argentatus*, *Numenius arquata*, *Capella gallinago*, *Lymnocyptes minimus*, *Tetrao urogallus*, *Phasianus colchicus*, *Perdix perdix*, *Gallus gallus*, *Columba livia*, *Accipiter nisus*, *A. gentilis*, *Asio otus*, *Emberiza citrinella*, *Pica pica* (11 specimens) and *Corvus corone cornix*.

The Sella turcica

All investigated birds have a deep and well ossified sella turcica (fig. 159). It is bounded rostrally by a transversal ridge which at the same time forms the ventro-medial circumference of the foramina optica. The lateral walls present a more or less extensive "shelf" for the n. oculomotorius near the top (fig. 160), and this shelf may run along the whole side of the sella or it may be restricted to its rostralmost parts. Laterally of this shelf there is a distinct ridge which separates the oculomotorius from the nervus trochlearis and the n. ophthalmicus (V) and this ridge is so high that it appears to be the lateral boundary of the sella. The caudal boundary of the sella is formed by a high dorsum sellae which projects rostro-dorsally from the floor of the brain-case. The dorsum is completely ossified in most larger birds, but is sometimes (e.g. specimens of *Columba*, *Phasianus*, *Accipiter gentilis*) penetrated by a foramen which lodges a connection between the sinus cavernosus and the vena basilaris medullae oblongatae mediana of Neugebauer (1845). Particularly in small birds but also in specimens of *Accipiter nisus*, *Numenius arquata* and *Pelecanoides* the dorsum is partly membraneous and only its dorsal margin has become ossified as a transversal bar, or this bar is also absent (e.g. specimens of *Elaenia*, *Leptastenthura*, *Capella*, *Lymnocyptes*). Pokorný 1926 described a transversal cartilaginous bar which separates the adeno-hypophysis

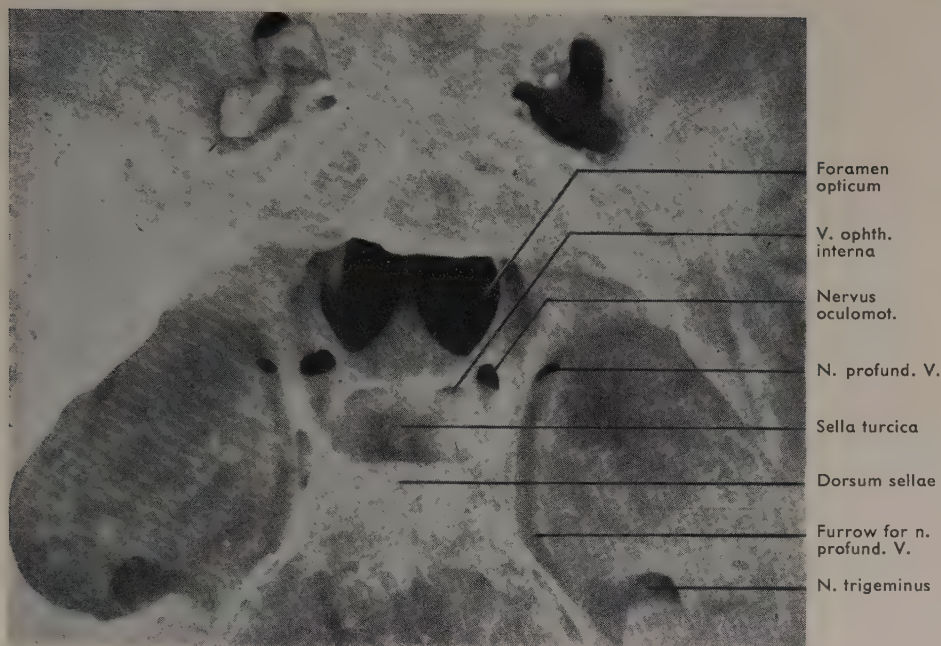


Fig. 159. *Pica pica*, ad. Photograph showing the sella turcica and the surrounding parts of the floor of the cavum cranii. The shelf for the nervus oculomotorius is distinct on both sides of the sella. The photograph is taken from a dorso-caudal direction. The foramina for the nerves and vessels are indicated. $6.5\times$.

from the neurohypophysis in "*Sylvia atra*" (probably *S. atricapilla*). In my single specimen this bar is present, but it is incomplete medially (absent in fig. 130). Pokorny also mentions that the lobes are separated by an ossified lamella in *Fringilla coelebs*. In one case (*Anser indicus*) the wall between the sella and the orbit is partly membranous, but in all other specimens the sella is completely separated from the orbit by bone.

The sella continues ventro-caudally as the two previously mentioned carotic channels, and just below the inferior margin of the foramen opticum there is a minute opening on each side which connects the sella with the orbit. This small foramen contains the arteria and vena ophthalmica interna and its external opening is just ventrolateral to the optic foramen and medial to the foramina for the eye muscle nerves and the n. profundus (ramus profundus V.). In *Emberiza* the channel for the vena ophthalmica interna and that of oculomotorius-abducens fuse before they open into the orbit.

An open canalis cranio-pharyngeus from the sella turcica to the ventral surface of the sphenoid was seen in some section series in which the epithelial stalk was partly or completely preserved (p. 33 ff.), and it could be studied particularly well in skulls of *Corvus* and *Pica*. It was completely continuous from the sella to the external surface of the sphenoid in three specimens of *Corvus corone cornix*.

and it is distinct also in 11 specimens of *Pica pica* though it is narrower and not completely continuous in all of them. The external aperture of this canalis cranio-pharyngeus is situated at the base of the rostrum sphenoidale just rostrally of the external apertures of the tubae Eustachii.

The Nerves of the Surroundings

The cranial nerve most intimately related to the pituitary is the oculomotorius which passes along the lateral margins of the sella on both sides of the eminentia and the infundibular stem (fig. 160). The nervus abducens regularly enters the bone behind the dorsum sellae on each side and passes through the pneumatic sinuses of the sphenoid laterally of the sella to the region of the optic foramen where it leaves the cranium together with or near the n. oculomotorius. On its way through the pneumatic sinuses of the sphenoid it is enclosed in an osseous tube and thus has no relations to the pituitary. In one adult specimen of *Riparia*, however, the only one sectioned with the pituitary in situ, the abducens is included in the sella and passes along the sides of the pituitary. The other nerves of the region, trochlearis, profundus and the ramus palatinus of the facialis, never enter into contact with the sella turcica or the pituitary. The small carotic nerve which was mentioned on p. 234, can be followed distinctly from its entrance along the carotis in embryos impregnated with silver. Its most important branch leaves the sella together with the a. ophthalmica interna and ends near the ganglion ciliare. The branch to the orbit can be seen sometimes also in the adult.

The Relations to Surrounding Vessels

The course of the carotis has been described on p. 264 ff. After forming the anastomosis in the caudal end of the sella it passes along the sides of the pituitary and then suddenly turns dorsally to penetrate the diaphragma sellae and enter the cavum cranii proper. The arteria ophthalmica, when present, continues rostrally to the end of the sella and leaves it through the foramen described above. A large volume in the sella is occupied by the sinus cavernosus, and the proportion of the sella occupied by the veins seems to be larger in the small birds. The vena ophthalmica interna is the direct rostral continuation of the sinus cavernosus on both sides and follows the a. ophthalmica (if present) through its foramen into the orbit. The vena nervi III never penetrates the dura mater but goes up to the latero-rostral edge of the sella outside this membrane and contacts the oculomotorius after the latter has penetrated the dura. It leaves the cranium together with the nerve.

The Relations to the Meninges

The dura mater in birds is attached to the periost of the neurocranium (v. Gelderen 1926). The part of the dura which covers the posterior surface of the dorsum sellae continues rostrally over the aperture of the sella as a diaphragma

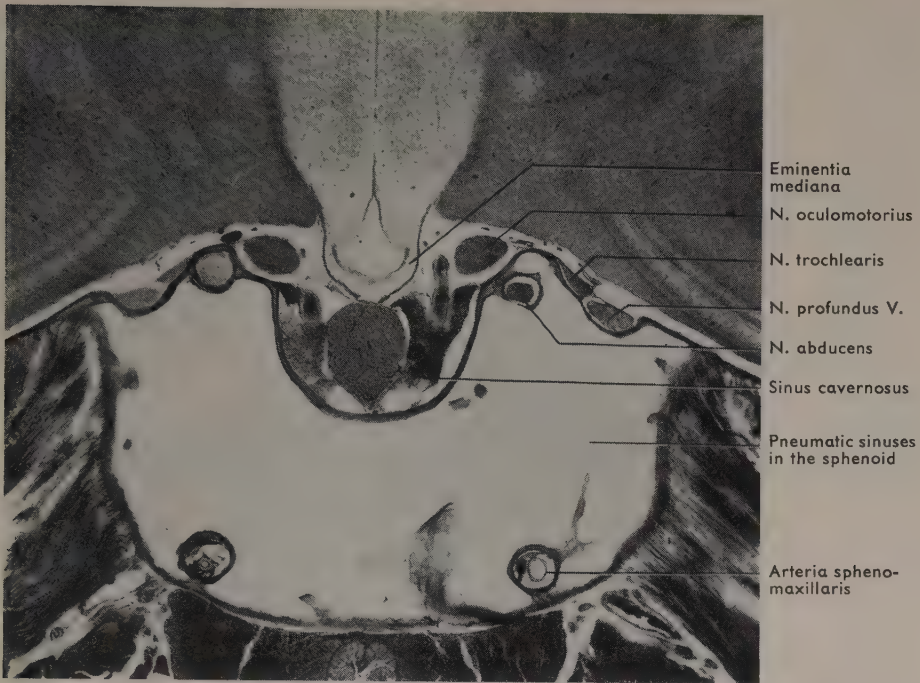


Fig. 160. *Emberiza citrinella*, ♀. Transversal section through the pituitary region. Note the large and uncomplicated sinus cavernosus. The carotid on the left (in the photograph right) side just penetrates the diaphragma sellae. — Bouin, celloidin 60 μ , phosphotungstic acid haematoxylin of Mallory. 22 $\times$.

sellae. The diaphragma extends rostrally to the region behind the chiasma and laterally of the oculomotorius and is continuous with the dura mater all around the sella. Just in front of the dorsum the diaphragma forms a fold into the sella in *Gallus* and some passerine birds. This fold extends between the dorsum sellae and the infundibular stem to obtain contact with the neural lobe (fig. 152). It contains the branches from the infundibular arteries which supply the neural lobe in these species. The arteries penetrate the diaphragma just at the bottom of the fold and enter the neural lobe directly.

The whole of the eminentia mediana is situated above this dura-diaphragma membrane and also the pars tuberalis proper is thus distinctly intradural. The portal zone starts in the subdural space, but penetrates the diaphragma and becomes extradural before it contacts the pars distalis, and the same course is followed by the portal vessels.

The infundibular stem penetrates the dura just in front of the dorsum sellae and the neural lobe is thus extradural. The carotids, finally, pass through a pair of separate foramina in the diaphragma on each side of the portal zone of the tuberalis, before they pass up to the brain (fig. 160).

The main part of the pituitary may thus be said to be extradural, and only the pars tuberalis proper and the eminentia mediana are distinctly intradural. The connective tissue elements investing the pars tuberalis are directly continuous with the pia mater, and the pars tuberalis thus appears to lie in the pia and is covered by it on both sides. The embryology shows that the primordium of the neural lobe is covered by a delicate intima piae, and this membrane is partly preserved in the adult, especially in species with a primitive neural lobe. The intima piae is then completed by additional layers of connective tissue between the blood vessels on the surface. Consequently there is a membrane corresponding to the pia around the neural lobe, but no subdural space or dura mater surrounds it. Since the arrangement of the membranes in the sella is so different from that in the brain-case proper, it may be justified not to use the term "pia" for the intrasellar parts, however. Haller v. Hallerstein (1934, p. 300), and v. Gelderen (1926) also arrived at the conclusion that the neural lobe and the pars distalis in birds are extradural. V. Gelderen is of the opinion that the periost of the sella represents the external portion (Endocranium) of his primitive external meninx (Ectomeninx), whereas the diaphragma sellae represents the internal portion (Dura). The two derivatives of the ectomeninx would, thus, be separated so as to include the sella turcica between them.

The connective tissue capsule of the adenohipophysis is often in direct contact with the diaphragma sellae dorsally, but in many species there is a thick layer of loose connective tissue between the two membranes. Only scattered venous vessels appear along this surface of the pars distalis, whereas the sides of it are directly bordered by the wide sinusoids of the sinus cavernosus. Ventrally the capsule of the pars distalis is in direct contact with the periost of the sella, and only a diffuse net of flattened veins separates the two membranes at some places (figure 144, also 2—5). The neural lobe is directly attached to the dorsum sellae but is surrounded laterally and often also ventrally by the large sinus cavernosus in this region.

Conclusion

In brief, the relation of the pituitary to the surroundings is as follows. The eminentia mediana and the pars tuberalis proper are intradural and are connected with the extradural parts in the sella by the infundibular stem and the portal zone of the tuberalis. The extradural parts are separated from the intradural ones by a diaphragma sellae which is continuous with the dura, and this membrane is penetrated by the stem, the carotids and by the portal zone of the tuberalis. The neural lobe and the pars distalis occupy only the median parts of the sella, and the remaining space is filled by the large carotids and the large venous vessels of the sinus cavernosus. It may be correct to say that the pituitary is suspended in a mighty stream of venous blood which comes from the orbit and, in some species at least, from the fore-brain, and leaves the sella through the vena carotis.

Surgical approach to the pars distalis or the neural lobe will invariably imply some disturbance in the venous circulation of the head and troublesome bleeding must be expected. It should be noted also, that the important venous pathway from the orbital region to the vena carotis may be destroyed by hypophysectomy.

Surgical approach to the intradural parts will be less troubled by large venous vessels, but other precautions are necessary here. Stalk section intending to denervate the neural lobe must leave the portal zone of the eminentia intact for else the blood supply to the pars distalis will be completely stopped. On the other hand, birds are a very favourable object for experimental research since they have a discrete portal zone with the portal vessels distinctly separate from the infundibular stem. It will thus be possible to ligate these vessels without damaging the neurohypophysis. These operations are, of course, possible only if a suitable way through the cranium can be found. The best one appears to be through the posterior wall of the orbit if an approach to the intradural parts is intended.

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